SYNTHESIS AND BIOLOGICAL PROFILE OF SOME NOVEL HETEROCYCLIC MOIETIES BEARING NITROGEN, SULPHUR AND OXYGEN ATOMS

A THESIS SUBMITTED TO
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IN
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BY

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UNDER THE GUIDANCE OF

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Statement under *O. Ph. D. 7* of Saurashtra University

The work included in the thesis is done by me under the supervision of Prof. Anamik K. Shah and the contribution made thereof is my own work.

Date:

Place: Dhawal N. Joshipura

Certificate

This is to certify that the present work submitted for the Ph. D. degree of Saurashtra University by Mr. Dhawal N. Joshipura has been the result of work carried out under my supervision and is a good contribution in the field of organic, heterocyclic and synthetic medicinal chemistry.

Date:

Place: Prof. Anamik K. Shah

DEDICATED TO

MY BELOVED MUMMY-PAPA

&

MY BELOVED SISTERS

ACKNOWLEDGEMENT

It is a moment of gratification and pride to look back with a sense of contentment at the long traveled path, to be able to recapture some of the fine moments, to be think of the infinite number of people, some who were with me from the beginning, some who joined me at different stages during this journey, whose kindness, love and blessings has brought me to this day. I wish to thank each of them from the bottom of my heart.

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Dhawal N. Joshipura
/01/2009, Rajkot

CONTENT

GENERAL REMARKS

1. Melting points were recorded by open capillary method and are uncorrected.
2. Infrared spectra were recorded on Shimadzu FT IR-8400 (Diffuse reflectance attachment) using KBr. Spectra were calibrated against the polystyrene absorption at 1610 cm^{-1}.
3. ^{1}H & ^{13}C NMR spectra were recorded on Bruker Avance II 400 spectrometer. Making a solution of samples in DMSO d_6 and $CDCl_3$ solvents using tetramethylsilane (TMS) as the internal standard unless otherwise mentioned, and are given in the δ scale. The standard abbreviations s, d, t, q, m, dd, dt, br s refer to singlet, doublet, triplet, quartet, multiplet, doublet of a doublet, doublet of a triplet, AB quartet and broad singlet respectively.
4. Mass spectra were recorded on Shimadzu GC MS-QP 2010 spectrometer operating at 70 eV using direct injection probe technique.
5. Analytical thin layer chromatography (TLC) was performed on Merck-precoated silica gel-G F_{254} aluminium plates. Visualization of the spots on TLC plates was achieved either by exposure to iodine vapor or UV light.
6. The chemicals used for the synthesis of intermediates and end products were purchased from Spectrochem, Sisco Research Laboratories (SRL), Thomas-Baker, Sd fine chemicals, Loba chemie and SU-Lab.
7. With solvents microwave assisted reactions were carried out in Qpro-M microwave synthesizer operating at 1000 W. While solvent less microwave assisted reactions were carried out in domestic microwave oven LG MS-192 W.
8. All evaporation of solvents was carried out under reduced pressure on Heidolph LABOROTA-400-efficient.
9. % Yield reported are isolated yields of material judged homogeneous by TLC and before recrystallization.
10. The structures and names of all compounds given in the experimental section and in physical data table were generated using ACD Chemsketch version 6.0.
11. Elemental analysis was carried out on Vario EL Carlo Erba 1108.

ABBREVIATIONS USED

MF	Molecular Formula
MW	Molecular Weight
MP	Melting Point
BP	Boiling Point
Sub.	Substitution
MW	Microwave
min.	Minute
hrs / h	Hours
Con. / con.	Concentrated
sec.	Second
i.e.	That is
e.g.	For example
viz	namely
RT	Room temperature
TLC	Thin Layer Chromatography
FT-IR	Fourier Transformed Infrared
NMR	Nuclear Magnetic Resonance
UV	Ultraviolet
GC-MS	Gas Chromatograph coupled with Mass Spectrometer
APT	Attached Proton Test
DEPT	Distortionless Enhancement Polarization Transfer
ABT	2-Amino Benzothiazole
SC	Side Chain
IMB	Isatin Mannich Base
AAC	Aryl Amino Coumarin
APH	Acetophenone Phenyl Hydrazone
PA	Pyrazole Aldehyde
TMS	Trimethylsilane
DMSO	Dimethylsulphoxide

DMF	Dimethylformamide
TEA	Triethylamine
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
VH	Vilsmeier–Haack
DDQ	Dicyclohexyldicarbodimide
BEMP	2-*tert*-butylimino-2-diethylamino-1, 3-dimethylperhydro-1, 3, 2-diazaphosphorine on polystyrene
MTT	3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide
CDK	Cyclin Dependent Kinases
AKT	Enzymes, member of serine / threonine-specific protein kinase family
FAK	Focal Adhesion Kinases
JNK	c-Jun N-terminal Kinases
LXR	Live-X-Receptor
HIV	Human Immunodeficiency Virus
HTLV	Human T-Lymphotropic virus
LAI	Leaf Area Index
LAV	Lymphadenopathy associated virus
EDF	Epidermal Growth Factor
PDGF	Platelers Derived Growth Factor
CNS	Central Nervous System
MAO	Monoaminooxidase
IC_{50}	Concentration for inhibiting growth of 50% organisms
MIC	Minimum Inhibitory Concentration
ED_{50}	Effective Dose 50
LD_{50}	Lethal Dose 50
ANP	Anti-Natriuretic Peptide
NMDA	*N*-methyl-D-aspartate
i. p.	Intraperitoneal
PTZ	Pentylenetetrazole
MPA	Mercapto Propionic Acid
ADT	After-Discharge Threshold

GABA	Gamma Amino Butyric Acid
MES	Maximal Electroshock
ScPTZ	Subcutaneous Pentylenetetrazole
ScSTY	Subcutaneous Strychnine
QSAR	Quantitative Structure Activity Relationship
SAR	Structure Activity Relationship
AchE	Acetylcholine Esterase
HRV	Human rhinovirus
3CP	3C Protease
HCV	Human corona virus
DNA	Deoxyribonucleic Acid
ATRA	All Trans Retinoic Acid
EAC	Ehrlich Ascites Carcinoma
GI	Gastrointestinal
SARS	Severe Acute Respiratory Syndrome
GPCRs	G Protein-Coupled Receptors
ADAMTS	ADAM metallopeptidase with thrombospondin type 1
RTK	Receptor Tyrosine Kinase
FGFr	Fibroblast Growth Factor receptor
VEGF	Vascular Endothelial Growth Factor
ATP	Adenosine Triphosphate
PTK	Protein Tyrosine Kinase
DHPM	Dihydropyrimidine
DBU	Diazabicycloundecene
DBN	Diazabicyclononene
EDG	Electrone Donating Group
EWG	Electrone Withdrawing Group
MRSA	Methicillin Resistance *Staphylococcus*
MRCNS	Methicillin Resistance Coagulase Negative *Staphylococci*
CAP	Community Acquired Pathogens
$POCl_3$	Phosphorous oxychloride
$ZnCl_2$	Zinc chloride

$ClCH_2COCl$	Chloroacetyl chloride
$AlCl_3$	Aluminium trichloride
EtOH	Ethanol
MeOH	Methanol
NaOH	Sodium hydroxide
HCl	Hydrochloric acid
K_2CO_3	Potassium carbonate
H_2SO_4	Sulphuric acid
KSCN	Potassium thiocyanate
BH_3.THF	Borane in tetrahydrofuran
KBr	Potassium bromide
$CDCl_3$	Deuteriated chloroform
$BF_3.Et_2O$	Borone trifluoride in diethylether
HCN	Hydrogen cyanide
TiCl4	Titanium tetrachloride
KOH	Potassium hydroxide
NaH	Sodium hydride
CaH_2	Calcium hydride
LiH	Lithium hydride
KF	Potassium fluoride
Al_2O_3	Aluminium trioxide
Br_2	Bromine
$FeCl_3$	Ferric chloride
$InCl_3$	Indium trichloride
$BiCl_3$	Bismuth trichloride
$LaCl_3$	Lanthanium trichloride
$LiClO_4$	Lithium perchlorate
VCl_3	Vanadium trichloride

PART – A

STUDIES ON 2-METHYL INDOLINE DERIVATIVES

A.1 INTRODUCTION TO INDOLE SYSTEM

Indole (2, 3-benzopyrrole, ketole, 1-benzazole; C_8H_7N) is an aromatic heterocyclic organic compound. It has a bicyclic structure, consisting of a six-membered benzene ring fused to a five-membered nitrogen-containing pyrrole ring. The participation of the nitrogen lone electron pair in the aromatic ring means that indole is not a base, and it does not behave like a simple amine.

Indole is a solid at room temperature. Indole can be produced by bacteria as a degradation product of the amino acid tryptophan. It occurs naturally in human feces and has an intense fecal odor. At very low concentrations, however, it has a flowery smell, and is a constituent of many flower scents (such as orange blossoms) and perfumes. It also occurs in coal tar.

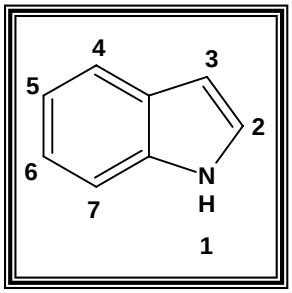

Fig. A.1

Many researchers have described synthesis of indole and its derivatives along with its applications in literature. [1-47]

A.1.1 PHYSICAL PROPERTIES OF INDOLE

Indole is a white coloured solid, melting at 52-54°C and boiling at 253-254 °C. 0.19 gm of indole is soluble in 100 ml of hot water. Indole is soluble in alchohol, ethylacetate etc. Indole is having planar molecular shape, 1.22 g/cm^3 density and 2.11 D dipole moment in benzene. All indole derivatives show certain family resemblances to indole [4], but striking changes can be brought about by substitution of groups in the pyrrole ring. Thus, the fecal-like

odor of skatole is the most pronounced of all the methylindoles, less pronounced for the 2-methylindole and the 2, 3-dimethyl indole; 1-methylindole, on the other hand, resembles methylaniline in odor. Introduction of carboxyl groups or phenolic hydroxyl groups causes elimination of the odor, and the naphthindoles are also without odor.

All the common indole derivatives, like indole, form well-defined crystalline picrates, yellow to red in color. Formation of picrates is usually a suitable procedure for identification and purification.

A.2 INTRODUCTION TO INDOLINE SYSTEM

Indoline (2, 3-dihydro-1*H*-indole; C_8H_9N) is an aromatic heterocyclic organic compound. It has a bicyclic structure, consisting of a six-membered benzene ring fused to a five-membered nitrogen-containing ring. The compound is based on the indole structure, but the C_2-C_3 bond is saturated by oxidation / dehydrogenation it can be converted to indoles.

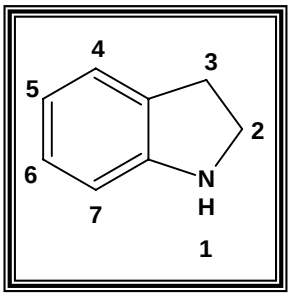

Fig. A.2

A.2.1 REDUCTION OF INDOLE

Under this title, only reduction of (un)substituted-1*H*-indole system is discussed, reduction of other indole analogues is not discussed as well. Many methods have been employed in the reduction of indoles, the nature of the product(s) depending upon the method used.

The indole nucleus is not reduced by sodium-amyl alcohol [48] or by sodium-butyl alcohol, [49] but indolines are produced by electrolytic reduction of

indoles in acid [50-52] and by reduction with phosphonium iodide in hydrogen iodide saturated hydriodic acid. [53]

1. *METAL-ACID REDUCTION*

The reduction of indole with zinc dust, [51] tin, [51, 54] or zinc amalgam [55] in hydrochloric acid affords indolines, but the yield of indoline produced from indole by such reductions is lowered by simultaneous polymerization of indole in the acidic media. This side reaction has been eliminated [56] by effecting this reduction with zinc dust in 85% phosphoric acid, under nitrogen to prevent aerial oxidation.

2. *BIRCH REDUCTION*

Early studies [62] led to the conclusion that sodium-ammonia reduction of indole affords indoline. However, the product from this reaction has been shown to be a mixture of indole and a product resulting from reduction in the benzenoid ring. [63] It was found that lithium-ammonia has very little reducing effect upon indole owing to the formation of the nonreducible indole lithium salt. [64-66]

3. *REDUCTIONS WITH SODIUM BOROHYDRIDE, LITHIUM ALUMINUM HYDRIDE, AND BORANE*

Indole is not reduced by either sodium borohydride [67] or lithium aluminum hydride, [67, 68] nor are other 1-unsubstituted indoles [69-70] reduced with lithium aluminum hydride. This nonreducibility has been illustrated in numerous cases in studies connected with the indole alkaloids where sodium borohydride [71] and lithium aluminum hydride [72] reductions of functional group(s) present in these complex molecules have been affected without reduction of the indole nucleus. Indole is reduced to indoline in 48% yield, however, by borane in tetrahydrofuran. [73] Although 1-methyl- and 1, 3-dimethylindole have been reported [68] to be reduced by lithium aluminum hydride to the corresponding indolines in 25-30% yields, later related studies [48] failed to support these observations. 1, 2, 3, 4-Tetrahydro-9-methylcarbazole remains unreduced when treated with lithium aluminum

hydride, [48] and 1, 3-disubstituted oxindoles afford 1, 3-disubstituted indoles upon reduction with this reagent.

4. *CATALYTIC HYDROGENATION*

Two early detailed studies on the catalytic hydrogenation of indoles were carried out. [74, 75] Whereas metal-acid reductions of indoles afford indolines as end products, catalytic hydrogenation of indoles often proceeds further than the indoline stage or occurs at positions alternative to the indolic 2, 3 double bond, and may even cause rupture of the pyrrolic ring. [76] Hydrogenation of indole with a nickel catalyst in ethanolic solution affords 1-ethyloctahydroindole by saturation of the aromatic system and reductive ethylation of the nitrogen atom. [77] Catalysts used for the hydrogenation of indoles to indolines are platinum, nickel, nickel salts, copper, copper salts, [76] and palladium hydroxide-barium sulfate. [78] It is interesting that under vigorous conditions using a copper chromite catalyst, hydrogenation of 2, 3-dimethylindole has been reported [59] to afford only *trans*-2, 3-dimethylindoline, the *cis* isomer being the expected sole product from this reaction. It is suggested [59] that under the vigorous conditions used the hydrogenation reaches equilibrium involving appreciable dehydrogenation of the indoline and thus the indoline formed is the more stable *trans* isomer. Presumably such an equilibration-dehydrogenation, if it occurs, would have to involve the formation of some 2, 3-dimethyl-3*H*-indole in order that it could ultimately effect the formation of the *trans*-indoline. However, the establishment of the *trans* configuration of the product in this work [59] leaves much to be desired and further investigation of this product would be of interest. 1, 2, 3, 4-tetrahydrocarbazole, [48] and its 9-methyl derivative [48, 75] afford, as expected, the *cis*-indolines upon catalytic hydrogenation.

2, 3-disubstituted indoles [57] have been hydrogenated to the corresponding 2, 3-disubstituted indolines in very high yields, often quantitative, using a platinum catalyst in ethanolic 42% w/w aqueous fluoroboric acid at room temperature and atmospheric pressure. It is claimed [57] that this procedure is extremely valuable since chemical reduction of indoles to indolines is often inconvenient and other methods of catalytic

hydrogenation of 2, 3-dialkylindoles often afford relatively much lower yields. It is essential, however, that the indole being hydrogenated must either be acid stable or be very rapidly reduced relative to being polymerized. [57]

When the above-mentioned hydrogenation of 1, 2, 3, 4-tetrahydro-9-methylcarbazole is continued, a second product is formed [79] which is probably dodecahydro-9-methylcarbazole. [79] Under the appropriate conditions indole, 2- and 3-methylindole, and 1, 2-dimethylindole can be catalytically hydrogenated to the corresponding octahydroindoles. [76] The A-B ring junction in such compounds is *cis*-fused as shown in Fig. A.3. [58-61, 80-84]

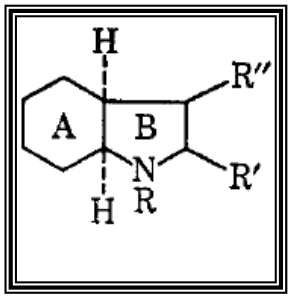

Fig. A.3

Several examples are known [49, 58, 85, 86] in which catalytic hydrogenation of an indole ring affords the 4, 5, 6, 7-tetrahydroindole derivatives. In one of these cases [85] an aliphatic nitro group in the molecule remains unreduced under conditions which would normally effect its conversion to an amino group.

5. *REDUCTIVE CLEAVAGE OF SUBSTITUENTS FROM THE INDOLE NUCLEUS*

2-ethoxyindole affords 66% of indole and 9.8% of indoline upon reduction with borane in tetrahydrofuran. [73] Similar cleavage of a 2-substituent occurs when compound (1) is exhaustively hydrogenated in the presence of a platinum catalyst to afford skatole and piperidine hydrobromide. [87] If this hydrogenation is arrested after the uptake of three moles of hydrogen or if it is affected in the presence of palladium-carbon instead of platinum, it affords compound (2). [87] (Fig. A.4)

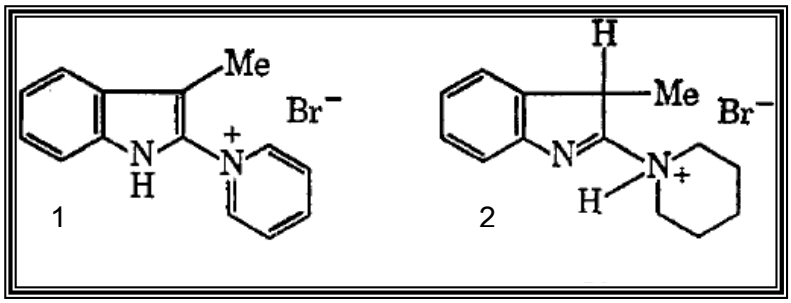

Fig. A.4

A.2.2 PREPARATION OF 2-METHYL INDOLINE

2-methyl indoline derivatives were reported to synthesize from corresponding indole derivatives using cyano sodium borohydride [88, 92] as a reducing agent and glacial acetic acid as a catalyst.

Kikugawa [89] produced 2-methyl indoline from 2-methyl indole using sodium borohydride, aluminium trichloride and pyridine as a catalyst.

Clive *et. al.* [90] reported preparation of 2-methyl indoline from 2-(phenylseleno) methyl indoline using triphenylstannane (Ph_3SnH).

Mills *et. al.* [91] reported different preparation methods for the 2-methyl indoline. (a) Treatment of ethyl acetoacetate with phenyl hydrazine and cyclization using sulfuric acid and ammonia and (b) From ethyl (2-allylphenyl) carbamate using benzene selenyl chloride and triphenylstannane.

Jackman and Scarmoutzos [93] synthesized 2-methyl indoline from 2-methyl indole using trimethylamine and borane.

Kotsuki *et. al.* [94] produced 2-methyl indoline by reducing 2-methyl indole using zinc borohydride and diethylether as a solvent.

Indoles were hydrogenated using heterogeneous catalysts in hydrocarbon solvents to achieve selective hydrogenation of the heterocyclic ring by Shaw and Stapp. [95] Hydrogenation of indoles using Pt, Re, or in some cases, Ni catalysts (with or without sulfur compounds) occurred exclusively in

the heterocyclic ring to give indolines, but conversions were affected by indole-indoline equilibrium.

The regioselective hydroamination and cyclization of aliphatic and aromatic amino olefins in the presence of $(LaHL_2)_2$ (L = η5-pentamethylcyclopentadienyl) [96] and borontrifluoride using diethylether and the divalent samarium complexes $Cp_2'Sm$ and $Cp_2'Sm(THF)_2$ (Cp' = η5-Me_5C_5) [97] and to give 2-methyl indoline was reported. Thus, $CH_2=CH(CH_2)_3NH_2$ was treated with a catalytic amount of $(LaHL_2)_2$ in a hydrocarbon solvent (toluene, cyclohexane or pentane) to give 2-methyl indoline. Kinetic and mechanistic evidence presented that the turnover-limiting step is intramolecular olefin insertion into the La-N bond followed by rapid protonolysis of the resulting La-C bond.

Lawin *et. al.* [98] reported the preparation of 2-methyl indoline from 2-methyl indole through electrolytic reduction.

Meyers and Melot [99] carried out *N*-alkylation on indoline followed by methylation at C_2 position in the presence of *t*-butyllithium and dealkylation using hydrazinehydrate resulted into corresponding indoline.

Yadav *et. al.* [100] reported that *N*-allyl anilines underwent 3-aza-Cope rearrangement in the presence of Zn^{+2} montmorillonite under microwave irradiation in the absence of solvent to afford indoline derivatives in high yields. Similarly aryl allyl thioethers were rearranged to dihydrobenzothiophenes.

Jimenez *et. al.* [101] reported mixtures of products, while 2-allyl aniline underwent reduction through beta-cyclodextrin medium.

N-methyl-2-methyl indoline was synthesized from *N*-methyl-2-methyl indole using tin and concentrated hydrochloric acid, [102] indium and ammonium chloride, [103] and from *t*-butyl 2-methyl-1*H*-indole-1-carboxylate using rhodium phosphine complex, [104] PhTRAP-ruthenium catalyst, [105] and palladium and polymethylhydrosiloxane. [106]

Furthermore, literature [107-118] revealed many synthetic procedures to synthesize 2-methyl indoline and its derivatives through cyclization and reduction.

A.2.3 *N*-ALKYLATION ON 2-METHYL INDOLINE

Indolines (1) (n = 1, 2, R = H, Me; NR^1R^2 = piperidino, morpholino, 2-methylpiperidino-1-indolinyl, tetrahydro-2-isoquinolyl; R^1 = R^2 = ethyl, propyl, isopropyl, etc.) were prepared by Sheinkman *et. al.* [119] in 18-96% yield by reaction of (2) with HNR^1R^2. (2) were prepared by reaction of $Cl(CH_2)nCOCl$ with the respective indoline. (3) was obtained by reaction of 1-(2-chloroethyl) piperidine and indole and also by reduction of (1) (R = H, n = 1, NR^1R^2 = piperidino) using $LiAlH_4$. (1) had weak anesthetic activity; (1) (R = H, n = 1, R^1 = R^2 = isopropyl) was the most active anesthetic; (1) (NR^1R^2 = piperidino, R = H, Me, n = 1, 2) were the most effective local anesthetics. (Fig. A.5)

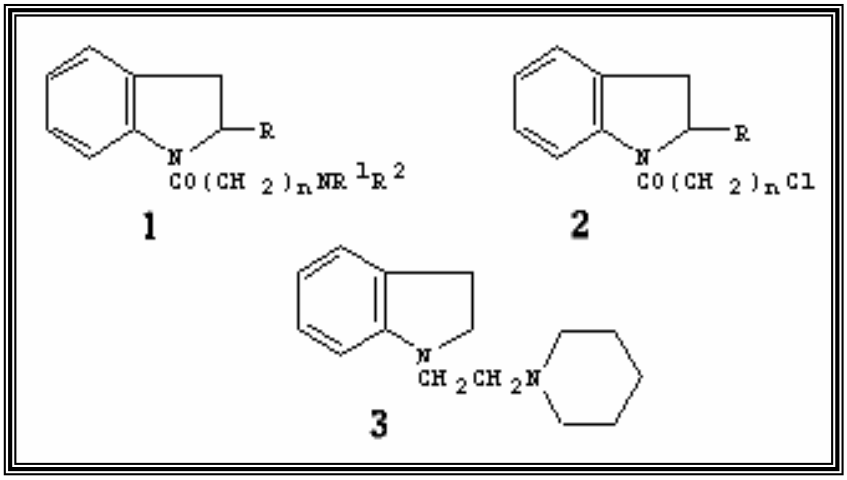

Fig. A.5

The title compounds (1) (R = diethylamino, morpholino; R^1 = R^3 = H; R^1R^3 = bond; R^2 = R^5 = H, methyl; R^4 = H, methyl, ethyl), (2), and (3) (R^6 = H, methyl; R^7 = diethylamino, morpholino) were prepared by Mutschler *et. al.* [120] by the reaction of diethylamine or morpholine with the corresponding chloroacetylindoles or chloroacetylindolines, which were prepared by the reaction of the parent compounds with chloroacetylchloride. (Fig. A.6)

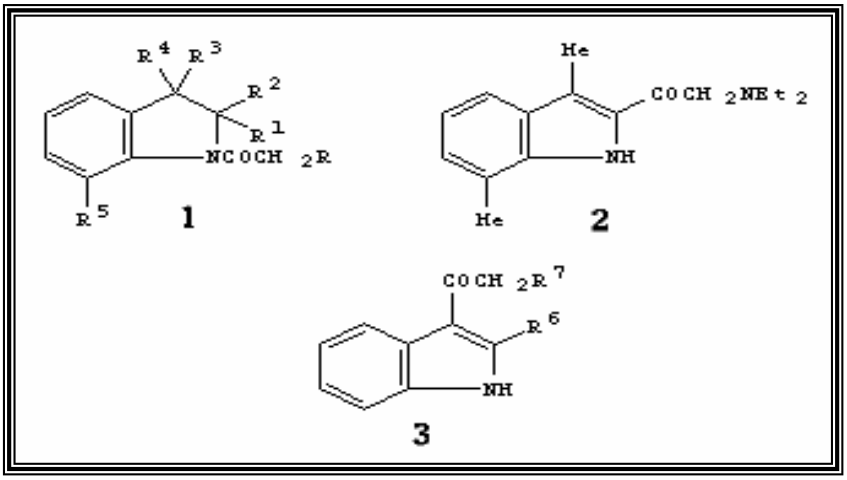

Fig. A.6

Alyab'eva *et. al.* [121] prepared Indolylbenzoates (1) (R^1 = H, Me; R^2 = H, MeO; R^3 = H, NO_2) in 40-92% yield by reaction of 5-substituted-2-chloro-benzoicacid with indoline (2) in the presence of K_2CO_3 and CuO. Cyclization of (1) (R^3 = H) with polyphosphoric acid gave 10-90% pyrroloacridinone (3). Treatment of (3) (R^1 = R^2 = H; R^1 = Me, R^2 = H) with MnO_2 gave 75-78% (4). Reaction of (3) (R^1 = R^2 = H) with $LiAlH_4$ gave 84% (5); while 71% (5) and 22% (6) were obtained on treatment of (3) (R^1 = Me, R^2 = H) with $LiAlH_4$. (Fig. A.7)

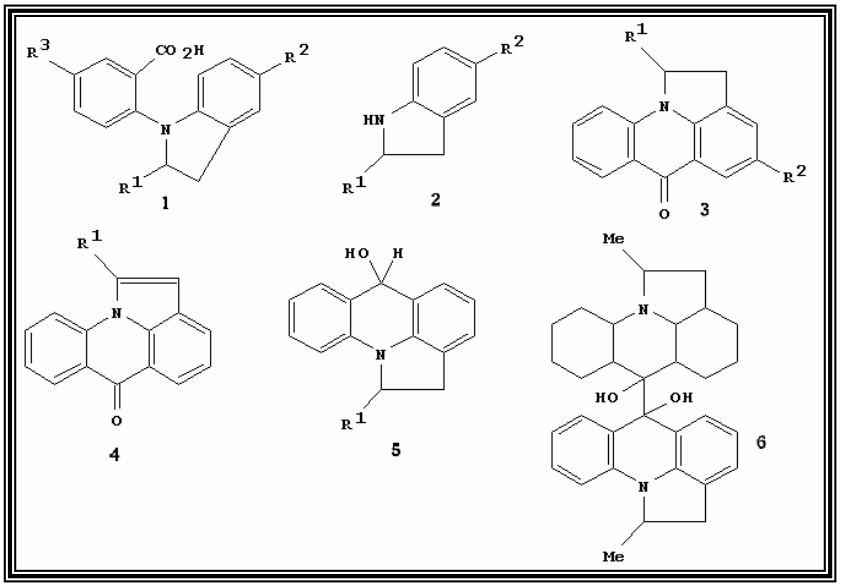

Fig. A.7

Kabbe *et. al.* [122] synthesized *N*-2-dimethyl indoline-1-carboxamide (alkylurea) derivatives from 2-methyl indoline using methyl isocyanate for the treatment of lipometabolic diseases / disorders.

Bermudez *et. al.* [123] reported that Indazole (1) is a potent and selective 5-HT_3 receptor antagonist. On those basis, a novel series of potent 5-HT_3 receptor antagonists, 1-indoline- and 1-indole carboxamides (2) [R = H , F, Cl, OCH_3, NO_2; R^1 = H, methyl, ethyl, phenyl; R^2 = H, methyl; R^1R^2 = $(CH_2)_2$, $(CH_2)_4$, $(CH_2)_5$; R^3 = H, methyl; n = 2, 3] and (3) (R^1 = H, methyl, ethyl, phenyl), respectively, were described. The activity of (2) suggested that aromaticity of the 5-membered ring was not an essential requirement for the potency provided that an in plane orientation of the CO group was favored. On the basis of this hypothesis indene (4) was prepared. In (4) the in plane orientation of the CO group was maintained by conjugation with the aromatic ring through the sp^2-hybridized C. (4) was also a potent 5-HT_3 receptor antagonist. (Fig. A.8 & 9)

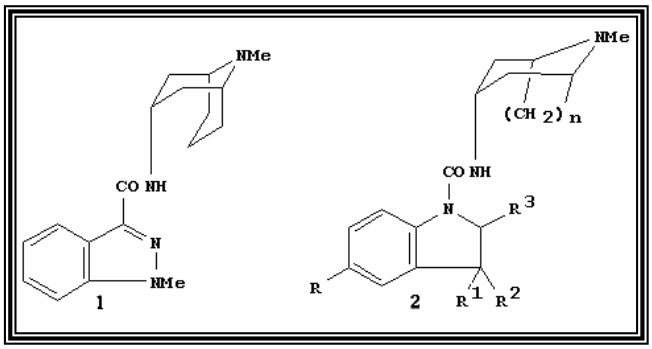

Fig. A.8

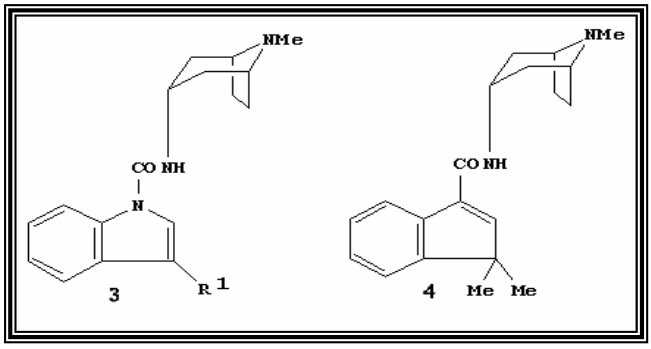

Fig. A.9

Spang *et. al.* [124] carried out formylation on 2-methyl indoline and prepared Schiff bases using differently substituted primary aromatic amines.

Indolines such as (1), (2), and (3) were prepared efficiently by Nicolaou *et. al.* [125] on solid supports by reaction of a resin-based selenyl bromide with substituted o-allylaniline derivatives to give resin-bound selenylmethyl indoline derivatives which could be functionalized and reductively cleaved to give methylindoline derivatives. The supported selenylmethyl indoline intermediates could either be cleaved directly by Bu_3SnH-mediated reduction to 2-methylindolines, acylated with phosgene and amines and reductive cleaved with Bu_3SnH to give methylindoline urea derivatives, or acylated with alkenoic acids or alkylated with allylic bromides to give amide and amine derivatives which underwent stereoselective radical cyclization upon resin cleavage to give polycyclic indolines. Treatment of o-allylaniline with resin-bound selenyl bromide in the presence of $SnCl_4$ gave a resin-bound selenylmethyl indoline; reduction of the resin-bound indoline with tributyltin hydride in the presence of AIBN gave (1) in 89% purity. Treatment of the resin-bound selenylmethyl indoline with a toluene solution of phosgene followed by addition of triethylamine and piperazine gave a resin-bound indoline urea derivative, which was acylated with 1-naphthalenecarboxylic acid and DCC and reductively cleaved with Bu_3SnH in the presence of AIBN to give piperazinylcarbonyl methylindoline (2) in 19% yield. Treatment of the resin-bound selenylmethyl indoline with $MeCH=CHCH_2Br$ and sodium hydride gave a resin-bound crotylindoline methylselenyl derivative which underwent stereoselective radical cyclization and cleavage to give tetrahydropyrroloindoline derivative (3) in 19% yield. The solid phase preparation of indolines cleavable by radical reduction allowed for the facile preparation of medicinally valuable methyl indoline derivatives and for the assembly of complex indoline natural product derivatives. Preparation of methyl indoline derivatives on solid phase allowed access to combinatorial libraries of structurally complex indoline derivatives. (Fig. A.10)

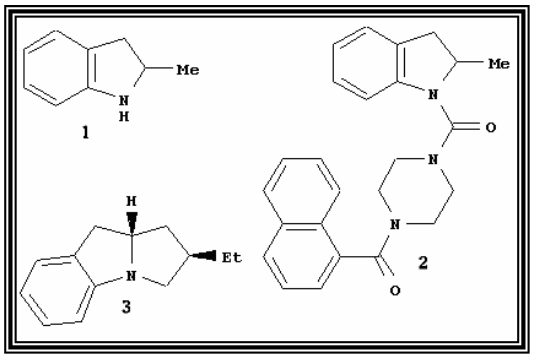

Fig. A.10

Ellis *et. al.* [126] replaced hydrogen atom of secondary amine in 2-methyl indoline with 1-bromo-9-chloroacridine using HMPT and carried out cytotoxic activity against 60 human cancer cell lines.

Furthermore using a polymer-bound selenenyl bromide resin, o-allyl and o-prenyl anilines were cycloloaded to afford a series of solid-supported indoline and indole scaffolds. [127] These scaffolds were then functionalized and cleaved via four distinct methods, namely traceless reduction, radical cyclization, radical rearrangement, and oxidative elimination, to afford 2-methyl indolines, e.g. (1), polycyclic indolines, e.g. (2), 2-methyl indoles, e.g. (3), and 2-propenyl indolines, e.g. (4), respectively. Numbers of small combinatorial libraries of compounds reminiscent of certain designed ligands of biological interest were constructed demonstrating the potential utility of the developed methodology to chemical and biological studies and the drug discovery process. (Fig. A.11)

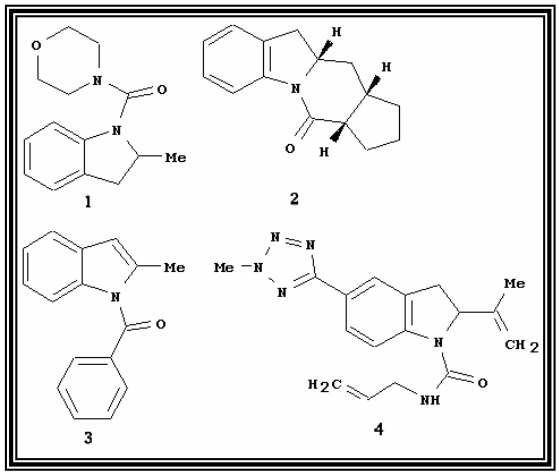

Fig. A.11

Zhao *et. al.* [128] optimized the lead compound 2-[-4-(4-chlorobenzyl)piperazin-1-yl]-1-(2, 3-dihydroindol-1-yl) ethanone by systematic structure-activity relation (SAR) studies and synthesized numbers of *N*-substituted (substituted-piperazinyl) 2-methyl indoline derivatives which led to the two potent compounds, 2 - [4 - (4 - chlorobenzyl) piperazine – 1 - yl] – 1 - (2 – methyl - 2, 3-dihydroindol – 1 - yl) ethanone and 2 - [4 - (4 - chlorobenzyl)piperazine – 1 - yl] – 1 - (2 – methyl - 2, 3 – dihydroindol – 1 - yl)ethanone as mixed D2/D4 receptor antagonists.

Tyunova *et. al.* [129] developed a convenient and efficient protocol for the synthesis of combinatorial library of 3-(2-thieno-sulfonyl) propionylamides. The synthetic route involved initial sulfochlorination of thiophene (or 2-bromothiophene), conversion of the resulting sulfochlorides into sulphinates followed by their reaction with acrylic acid. The resulting thienosulfonylpropionic acids were converted into the corresponding acid chlorides, which were used for acylation of primary and secondary aliphatic, aromatic and heteroaromatic amines. Several physico-chemical molecular parameters were calculated for the synthesized compounds, related to their potential pharmacokinetic profile.

Bordon *et. al.* [130] synthesized new 2-methyl indoline derivatives (1) [Y = N, O, S, CHR^3, CR^3; the dotted lines = single or double bond; R, R^1 = H, halo, OH, alkyl, alkoxy, CN, NO_2, NR^4R^5, CF_3, CF_3O, aryl, heteroaryl, $S(O)nNR^4R^5$; n = 0 - 2; R^3 = H, halo, alkyl, CN, NO_2, NR^4R^5, CF_3, aryl; R^2 = R^4, OR^4, SR^4 or NR^4R^5; R^4 = H, alkyl, cycloalkyl, aryl; either R^4 and R^5 is selected among the values of R^4 or heterocyclic containing N, O and S, all optionally substituted], these products being in all the isomer forms - racemates, enantiomers or diastereomers - and pharmaceutically acceptable salts, for use as drugs. Thus, trans-*N*-[6-(5, 6-dichloro-1*H*-benzimidazol-1-yl) 9*H*-purin-2-yl]-1, 4-cyclohexanediamine (2-HCl) was prepared, from 2, 6-dichloropurine via amination with 5, 6-dichloro-1*H*-benzimidazole in butanol followed by fusion with trans-1, 4-diaminocyclohexane. The protein kinase inhibitory activity of (2) as hydrochloride was detected [IC_{50} = 1.3 μM vs CIV-CDK; 98% inhibition SRC kinase at the rate 20 μM; 93% inhibition CDK1 at the rate 20 μM; 98%

inhibition ZAP kinase at the rate 20 μM; 93% inhibition casein kinase (2) at the rate 20 μM; 100% inhibition AKT kinase at the rate 20 μM; IC_{50} = 2 μM vs FAK kinase; IC_{50} = 0.84 μM vs JNK3 kinase]. (Fig. A.12)

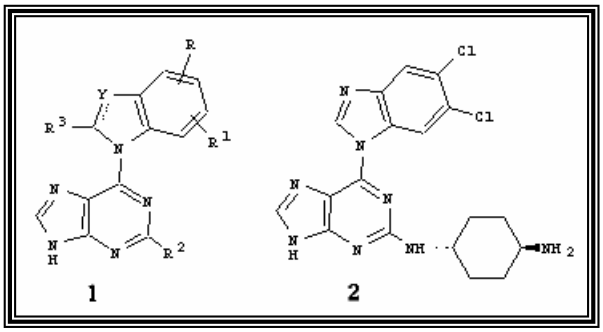

Fig. A.12

Gonzales *et. al.* [131] synthesized different *N*-(4-sulfamoylphenyl) amide derivatives of 2-methyl indoline as voltage-gated sodium channels inhibitors.

Dehmlow *et. al.* [132] reported preparation of indolyl hexafluoropropanols as Live-X-Receptor (LXR) modulators for the treatment of diabetes and related diseases. The invention relates to compounds (1) [wherein R^1 - R^6 = H, alkyl, etc.; A = (un)substituted aryl or heterocyclyl; m, p = 0-3; n = 0 or 1; R^3 and R^4 are absent when a is a double bond, with limitations, and pharmaceutically acceptable salts and esters thereof], their pharmaceutical compositions, processes for their preparations, and their use in the treatment and prophylaxis of diseases modulated by LXRα and/or LXRβ agonists, such as diabetes. For instance, (2), which showed IC_{50} values of 0.02 μM and 0.006 μM against LXRα and LXRβ, respectively, in the binding assay, was synthesized in multiple steps from 2-methyl-2,3-dihydro-1*H*-indole, hexafluoroacetone sesquihydrate and methyl-3-(chloromethyl) benzoate. (Fig. A.13)

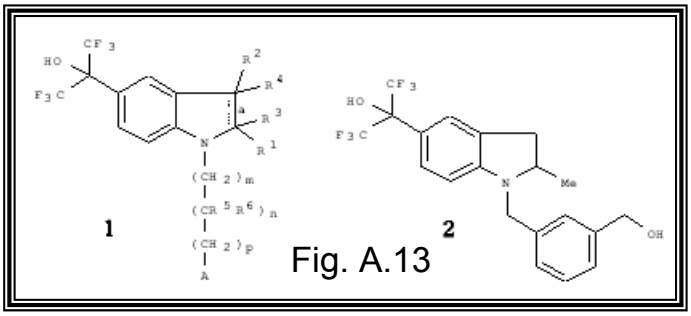

Fig. A.13

Literature also revealed different synthetic methodologies [133-143] for the synthesis of *N*-substituted-2-methyl indoline derivatives.

A.2.4 MANNICH REACTION ON 2-METHYL INDOLINE

Abonia *et. al.* [144] in their effort to synthesize pyrroloquinolines, synthesized 1-(benzotriazol-1(2)-ylmethyl)indolines for which they carried out Mannich reaction on 2-methyl indoline using benzotriazole as a secondary amine, formaldehyde and diethylether as a solvent and stirred for 30 minutes at room temperature. This Mannich base was reacted with unactivated and electron-rich alkenes in the presence of p-toluenesulfonic acid catalyst to give pyrroloquinolines but the pharmacological importance of the synthesized molecules was not reported.

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CHAPTER – 1

PREPARATION AND YIELD OPTIMIZATION OF 2-METHYL INDOLINE AND STUDY OF MANNICH REACTION ON 2-METHYL INDOLINE MOIETY

1.1 AIM OF CURRENT WORK

Since last few years, our group is involved in the synthesis of nitrogen containing heterocycles *viz*. pyrrole, indole, 2-methyl indole, dihydropyridine, dihydropyrimidine, 4-hydroxy quinolones etc. Where, pyrrole, indole, dihydropyridine, dihydropyrimidine, 4-hydroxy quinolone and 2-methyl indole showed good anti tubercular, anti diabetic, anti cancer and multi drug resistance reversal activity. Looking to the interesting biological profile showed by indole, 2-methyl indole and 2-methyl indoline from the literature survey and development of a simple preparation method for 2-methyl indole by our group we decided to prepare 2-methyl indoline and to explore the chemistry involving 2-methyl indoline moiety.

Literature revealed that different types of *N*-alkylation reactions have been carried out on 2-methyl indoline which include introduction of acetyl group, introduction of chloroacetyl group and further treatment with secondary amines, formylation at N_1 position and preparation of Schiff bases, preparation of amide linkages and alkylation by means of one and two carbon chains. Recently [a] Mannich reaction has been carried out on N_1 position in 2-methyl indoline using benzotriazole and formaldehyde. Looking to the reactivity of N_1 position for Mannich reaction, the secondary nitrogen is more active than C_3 while in 2-methyl indole, Mannich reaction goes on both N_1 and C_3 positions depending upon the reaction conditions.

Mannich bases can be synthesized by Mannich reaction on nitrogen of secondary amine having hydrogen atom with pronounced activity using simplified methodology and easy work up and this inspired us to develop some new *N*-substituted 2-methyl indoline derivatives by Mannich reaction. Literature also revealed that secondary amines *viz.* morpholine, piperidine, pyrrolidine, piperazine derivatives and other secondary amines like dimethylamine, diethylamine etc. and primary and secondary aromatic amines have not been used yet that is why we used primary and secondary amines to

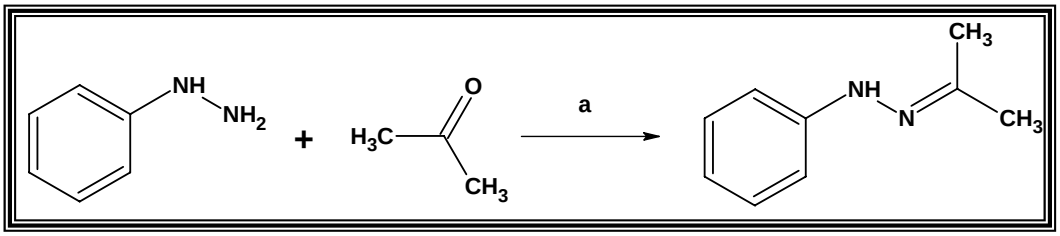
[a] R. Abonia, A. Albornoz, B. Insuasty, J. Quiroga, H. Meier, A. Hormaza, M. Nogueras, A. Sanchez, J. Cobo and J. N. Low; *Tetrahedron*, **2001**, *57(23)*, 4933.

acquire Mannich bases having desired scaffolds. These interesting Mannich bases derived from 2-methyl indoline are not only structurally novel but the biological evaluation is reported here for the first time. Biological importance of such an important scaffold is the rational behind the current work done in this chapter.

1.2 REACTION SCHEMES

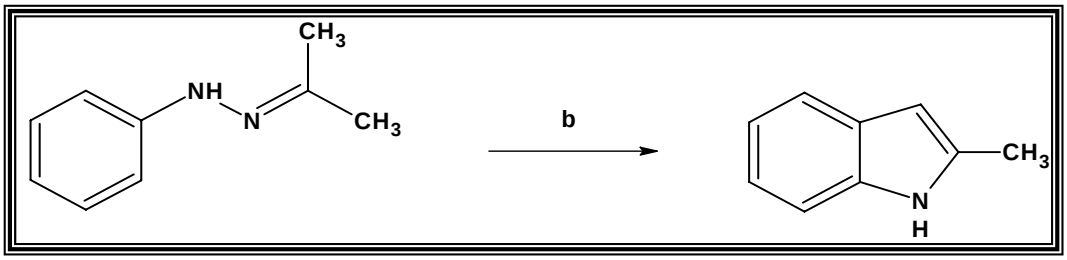
1.2.1 PREPARATION OF 2 – METHYL INDOLINE

STEP – 1



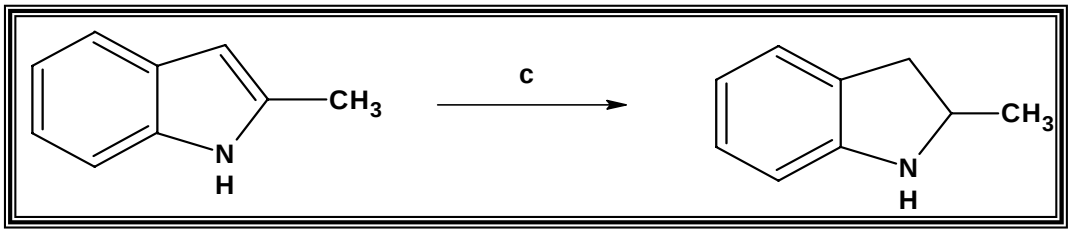
Reagents / Reaction Condition (a): Phenyl hydrazine, Acetone / Addition at RT.

STEP – 2



Reagents / Reaction Condition (b): Polyphosphoric acid / 120°C.

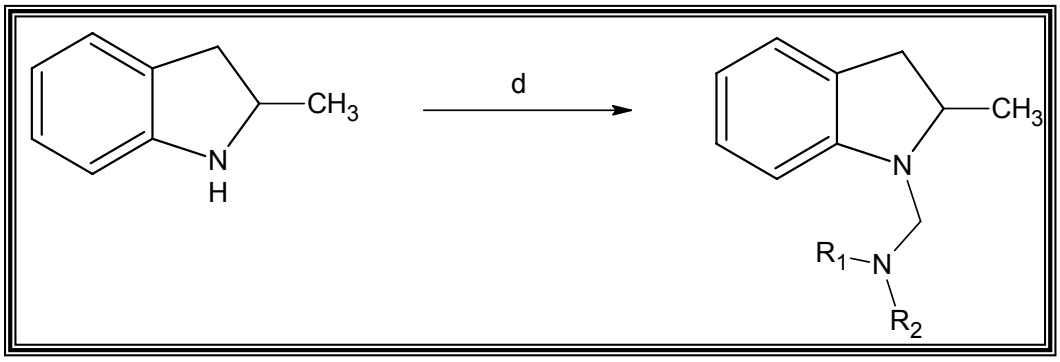
STEP – 3



Reagents / Reaction Condition (c): TFA, 1 M BH_3.THF / 0-5°C. (Method – A)
Zinc dust, 85% phosphoric acid. (Method – B)

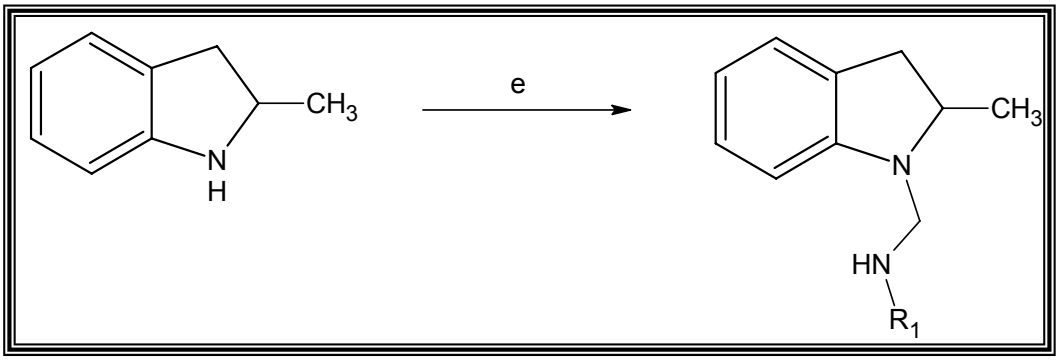
1.2.2 PREPARATION OF 2-METHYL INDOLINE *N*-MANNICH BASES

(1)



Reagents / Reaction Condition (d): Secondary amines, Diethylether, Formaldehyde (37-41%), Con. HCl / RT, stirring.

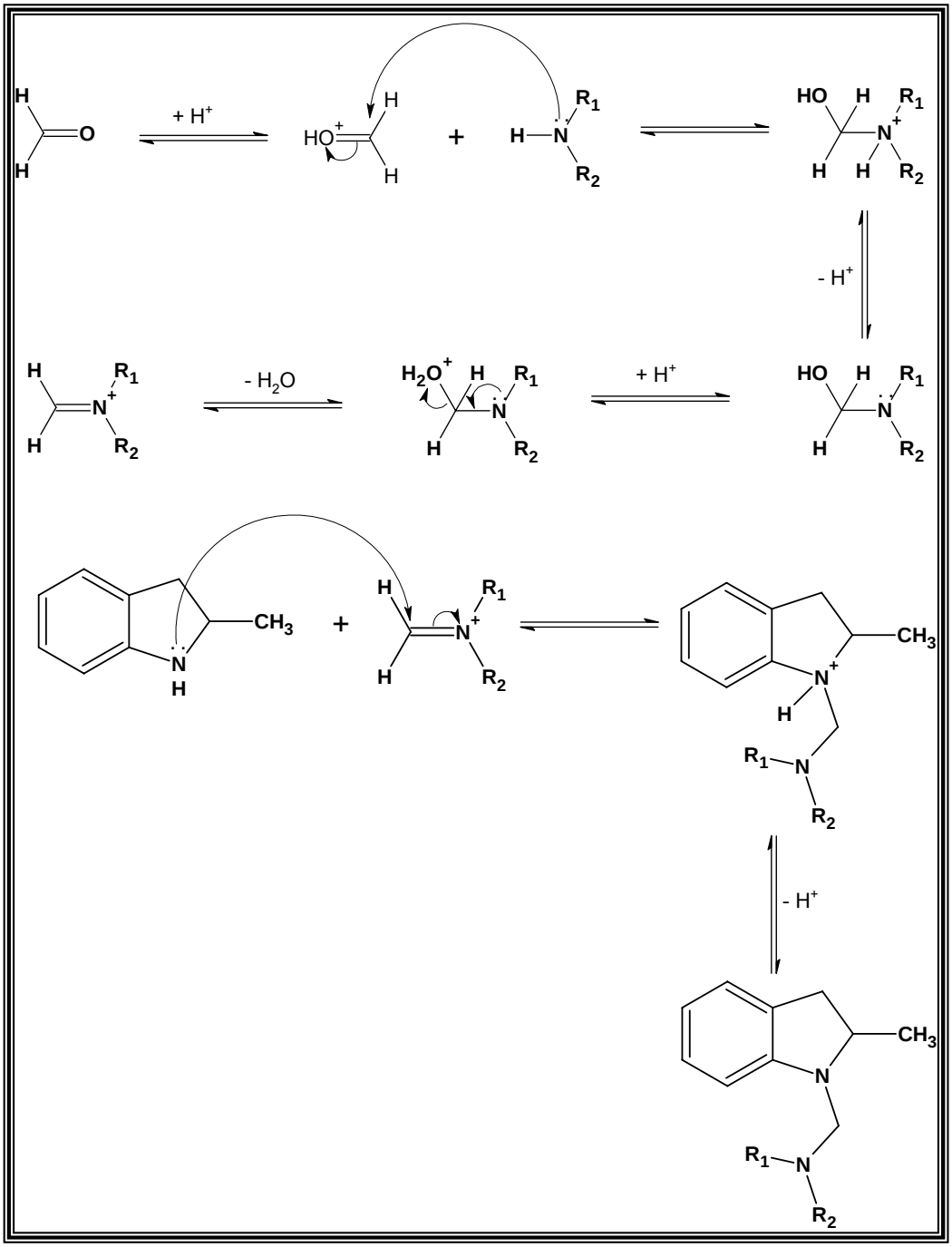
(2)



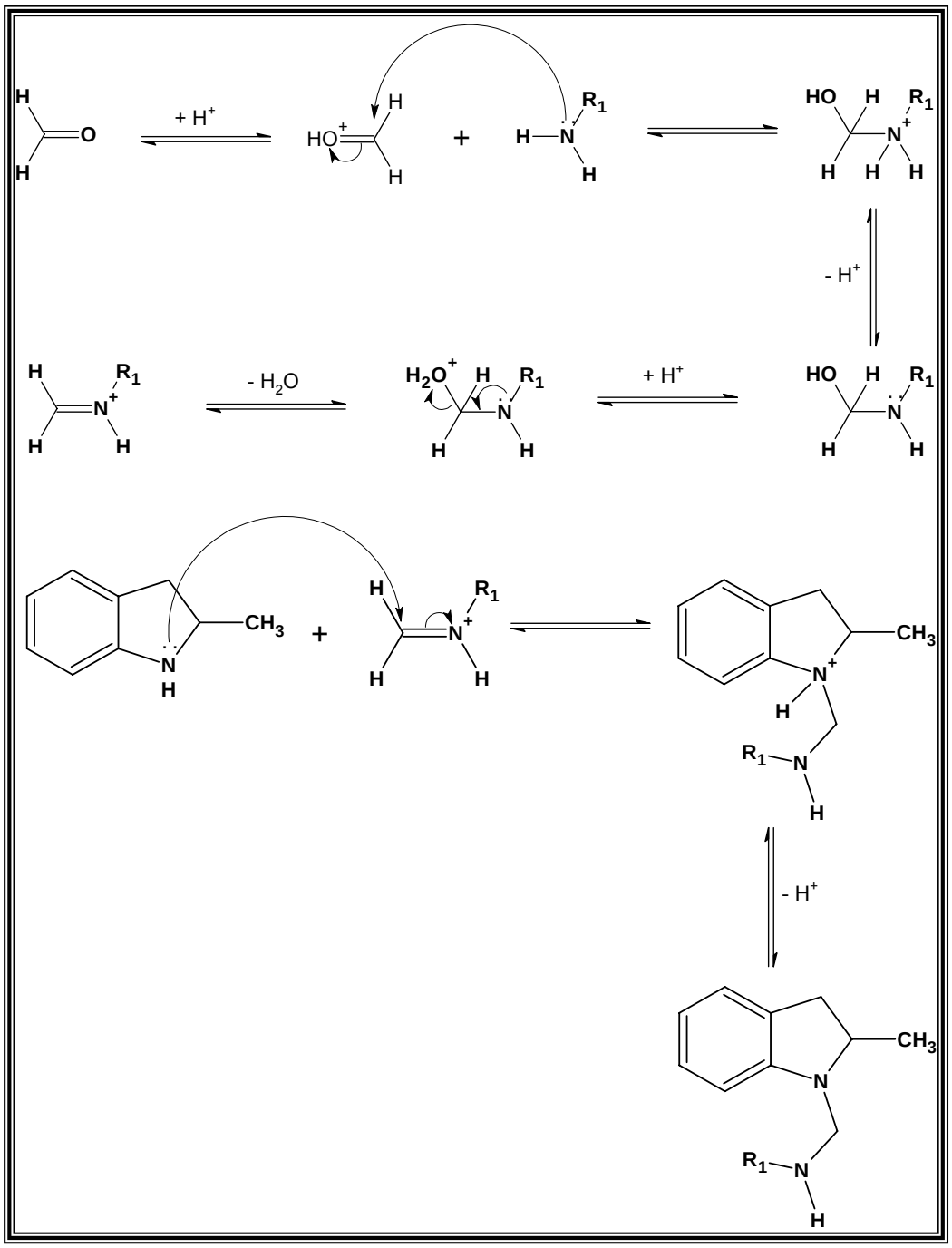
Reagents / Reaction Condition (e): Primary amines, Diethylether, Formaldehyde (37-41%), Con. HCl / RT, stirring.

1.3 PLAUSIBLE REACTION MECHANISM

1.3.1 SCHEME - 1



1.3.2 SCHEME - 2



1.4 EXPERIMENTAL

1.4.1 PREPARATION OF 2 – METHYL INDOLINE

STEP – 1 PREPARATION OF ACETONE PHENYL HYDRAZONE

25 ml of phenyl hydrazine was added drop wise to a magnetically stirred solution of 20 ml of acetone. After the completion of the addition, 5 ml of acetone was added to the reaction mixture and the reaction mixture was heated on the water bath to remove the excess of the acetone. Afterwards the reaction mixture was cooled to room temperature and it was made anhydrous by means of anhydrous sodium sulphate or anhydrous calcium chloride. The solution was filtered to give the dark yellow solution of phenyl hydrazone. Yield - 80 %, BP - 140-142°C (141-142°C [b])

STEP – 2 PREPARATION OF 2-METHYL INDOLE

30 gm of acetone phenyl hydrazone was added drop wise to a beaker containing 75 gm of polyphosphoric acid with constant stirring. The reaction mixture was heated on water bath for 2-3 hours, where the orange coloured solution became dark red-brown. After that the temperature of the reaction mixture was raised to 120°C and then it was cooled to room temperature. After that 400 ml of distilled water was added to the reaction mixture to decompose the polyphosphoric acid, the whole content was steam distilled to acquire the 2-Methyl Indole as white coloured shining crystals. Yield - 79 %, MP - 58-59°C (56-57°C [c])

STEP – 3 PREPARATION OF 2-METHYL INDOLINE

METHOD – (A)

0.05 mole 2-methyl indole was dissolved in 110 ml of trifluoroacetic acid under nitrogen atmosphere. The solution was cooled in an ice bath and 90 ml of about 1 M BH_3.THF in tetrahydrofuran solution was added slowly

[b] H. M. Kissman, D. W. Farnsworth and B. Witkop; *J. Am. Chem. Soc.*, **1952**, *74*, 3948.

[c] C. F. H. Allen and J. Vanallan; *Organic Syntheses*, **1955**, *Coll. Vol. 3*, p. 597.

over about 30 minutes. Thereafter, 50 ml of water was added, the resulting solution was stirred at room temperature for about 90 minutes. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using toluene : ethyl acetate (7 : 3) as a mobile phase. After the reaction to be completed the mixture was then evaporated under reduced pressure to about 30 ml of semi-solid viscous oil. The oil was partitioned between methylene dichloride and aqueous sodium hydroxide solution (pH>10). The organic layer was dried over anhydrous potassium carbonate, filtered and evaporated under reduced pressure to obtain 5.65 gm of a slightly greenish transparent oily 2-methyl indoline product. Yield - 85%, BP – 224-226 °C (225-227°C [d])

REVERSE ADDITION METHOD:

0.05 mole 2-methyl indole was dissolved in 90 ml of 1 M BH_3.THF solution in tetrahydrofuran by stirring under nitrogen atmosphere while cooling in an ice bath and the stirring was continued for about 15 minutes. Thereafter, 110 ml of trifluoroacetic acid was added drop-wise with continued stirring, cooling and maintaining a nitrogen atmosphere. 50 ml of water was added to the mixture, followed by methylene dichloride and aqueous sodium hydroxide solution (pH>10). The solution then was stirred about 30 minutes and a sample was taken for TLC, the progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using toluene : ethyl acetate (7 : 3) as a mobile phase. The organic layer was dried over anhydrous potassium carbonate, filtered and evaporated under reduced pressure to obtain 5.70 gm of a slightly greenish transparent oily 2-methyl indoline product. Yield - 86%, BP – 224-226 °C (225-227°C [d])

METHOD – (B)

To 0.36 mole of untreated zinc dust in a nitrogen atmosphere was added all at once 200 ml of 85 % phosphoric acid. The mixture was rapidly heated to 70-80°C on a steam bath and 0.12 mole of 2-methyl indole was added in portions during 30 minutes with vigorous mechanical stirring

[d] J. T. Shaw and F. T. Ryson; *J. Am. Chem. Soc.*, **1956**, *78*, 2538.

(efficient mechanical stirring was found to be extremely difficult if the mixture was kept at room temperature during the addition of indole, and the yields of indoline were only 5-15 %). After the addition to be completed stirring was continued for 3-4 hours at 80°C under nitrogen. 100 ml water was added with cooling and the mixture was basified slowly with 40% aqueous sodium hydroxide. The thick slurry was steam distilled and the extraction of the distillate with chloroform gave, after drying and concentration in *vacuuo*, greenish oil. Distillation under reduced pressure gave 65 % of 2-methyl indoline as slightly greenish transparent oil. BP – 224-226 °C (225-227°C [d])

1.4.2 PREPARATION OF 2-METHYL INDOLINE MANNICH BASES

(1) GENERAL PROCEDURE FOR THE PREPARATION OF DNJ-101 TO DNJ-111

It was prepared according to the method described by Abonia *et. al.* [a] 0.01 mole of 2-methyl indoline was charged into 50 ml erlenmeyer flask and 0.015 mole of formaldehyde (37-41% w/w solution) and 10 ml diethylether were added into above flask and the mixture was magnetically stirred for some time at room temperature under acidic condition. 0.01 mole of an appropriate secondary amine was added drop wise into above reaction mixture. Stirring was continued for further half an hour at room temperature. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using hexane : ethyl acetate (9 : 1) as a mobile phase. After the reaction to be completed, the reaction mixture was extracted using ethyl acetate (30 ml X 3). The combined organic layer was washed using water (20 ml X 2). The organic layer was dried over anhydrous sodium sulphate and the solvent was removed under reduced pressure to acquire the product. (Physical data of the synthesized end products are summarized in the table 1.5.1)

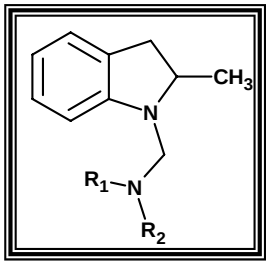
(2) GENERAL PROCEDURE FOR THE PREPARATION OF DNJ-201 TO DNJ-210

It was prepared according to the method described by Abonia *et. al.* [a] 0.01 mole of 2-methyl indoline was charged into 50 ml erlenmeyer flask and

0.015 mole of formaldehyde (37-41% w/w solution) and 10 ml diethylether were added into above flask and the mixture was magnetically stirred for some time at room temperature under acidic condition. 0.01 mole of an appropriately substituted primary amine was added drop wise into above reaction mixture. Stirring was continued for further half an hour at room temperature. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using hexane : ethyl acetate (9 : 1) as a mobile phase. After the reaction to be completed, the reaction mixture was extracted using ethyl acetate (30 ml X 3). The combined organic layer was washed using water (20 ml X 2). The organic layer was dried over anhydrous sodium sulphate and the solvent was removed under reduced pressure to acquire the product. (Physical data of the synthesized end products are summarized in the table 1.5.2)

1.5 PHYSICAL DATA TABLES

1.5.1 Physical data of *N*-substituted 2-methyl indoline *N*-Mannich bases (DNJ-101 to DNJ-111)

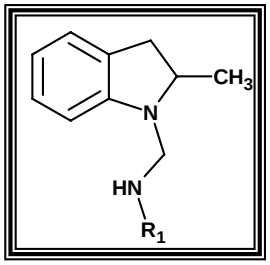


Code	Substitution R_1 & R_2	MF	MW (g/m)	R_f	% Yield
DNJ-101	Piperidine	$C_{15}H_{22}N_2$	230	0.54	55
DNJ-102	Morpholine	$C_{14}H_{20}N_2O$	232	0.52	61
DNJ-103*	Piperazine	$C_{14}H_{21}N_3$	231	0.53	75
DNJ-104	1-methyl piperazine	$C_{15}H_{23}N_3$	245	0.55	77
DNJ-105	1-ethyl piperazine	$C_{16}H_{25}N_3$	259	0.57	65
DNJ-106	Pyrrolidine	$C_{14}H_{20}N_2$	216	0.51	50
DNJ-107	1-benzyl piperazine	$C_{21}H_{27}N_3$	321	0.63	79
DNJ-108	*N*, *N*-dimethyl amine	$C_{12}H_{18}N_2$	190	0.49	67
DNJ-109	*N*, *N*-diethyl amine	$C_{14}H_{22}N_2$	218	0.52	56
DNJ-110	*N*, *N*-dipropyl amine	$C_{16}H_{26}N_2$	246	0.56	72
DNJ-111	*N*, *N*-dibutyl amine	$C_{18}H_{30}N_2$	274	0.60	49

R_f value was calculated using solvent system = Hexane : Ethyl Acetate (9 : 1)

* MP = 160-162°C

1.5.2 Physical data of *N*-substituted 2-methyl indoline *N*-Mannich bases (DNJ-201 to DNJ-210)



Code	Substitution R_1	MF	MW (g/m)	R_f	% Yield
DNJ-201	Aniline	$C_{16}H_{18}N_2$	238	0.56	61
DNJ-202	3-chloro aniline	$C_{16}H_{17}ClN_2$	272	0.58	55
DNJ-203	3-trifluoromethyl aniline	$C_{17}H_{17}F_3N_2$	306	0.58	77
DNJ-204	3-methyl aniline	$C_{17}H_{20}N_2$	252	0.58	75
DNJ-205	3-methoxy aniline	$C_{17}H_{20}N_2O$	268	0.59	50
DNJ-206	*N*-methyl aniline	$C_{17}H_{20}N_2$	252	0.57	65
DNJ-207	*N*, *N*-diphenyl amine	$C_{22}H_{22}N_2$	314	0.64	67
DNJ-208	3-amino pyridine	$C_{15}H_{17}N_3$	239	0.55	79
DNJ-209	4-amino pyridine	$C_{15}H_{17}N_3$	239	0.54	72
DNJ-210	Furfuryl amine	$C_{15}H_{18}N_2O$	242	0.53	56

R_f value was calculated using solvent system = Hexane : Ethyl Acetate (9 : 1)

1.6 SPECTRAL DISCUSSION

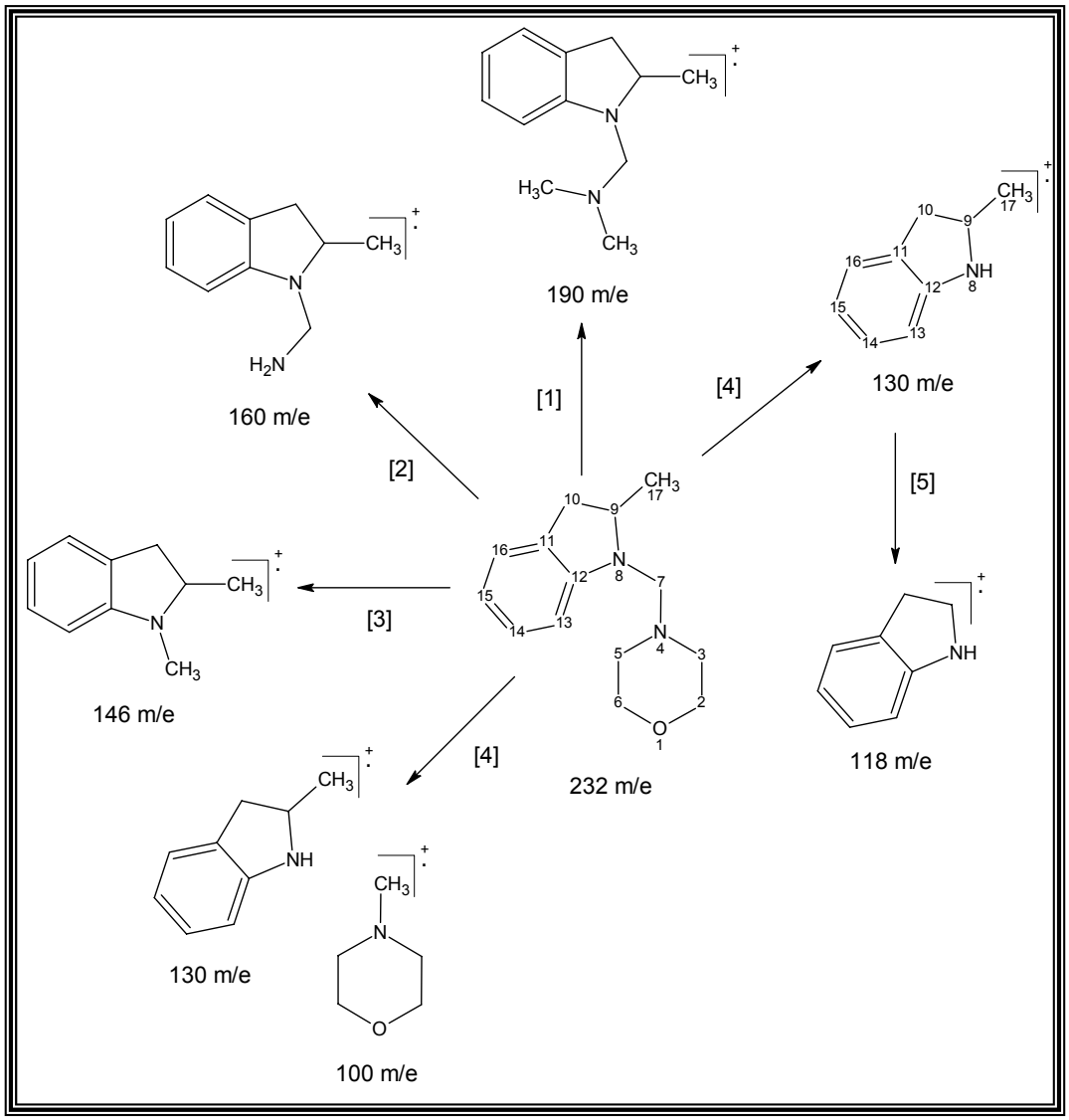
1.6.1 MASS SPECTRAL STUDY

Mass spectra of the synthesized compounds were recorded on **Shimadzu GC-MS QP-2010** model using direct injection probe technique. The molecular ion peak was found in agreement with molecular weight of the respective compound. Characteristic M^{+2} ion peaks with one-third intensity of molecular ion peak were observed in case of compounds having chlorine atom. Fragmentation pattern can be observed to be particular for these compounds and the characteristic peaks obtained for each compound. Probable fragmentation pattern for DNJ-102 and DNJ-206 can be discussed as under.

2-methyl-1-(morpholin-4-ylmethyl) indoline (DNJ-102)

1. The target compound showed characteristic molecular ion peak.
2. C_2-C_3 and C_5-C_6 bond cleavage gave characteristic peak at 190 m/e. [1]
3. C_3-N_4 and C_5-N_4 bond cleavage gave characteristic peak at 160 m/e. [2]
4. N_4-C_7 bond cleavage gave characteristic peak, which is the BASE PEAK at 146 m/e. [3]
5. C_7-N_8 bond cleavage gave two characteristic peaks. One peak at 130 m/e and second peak at 100 m/e , which is the second intense peak in the spectrum. [4]
6. After cleaved from bond C_7-N_8, C_9-C_{17} bond cleavage gave characteristic peak at 118 m/e. [5]

1.6.1.1 FRAGMENTATION PATTERN FOR DNJ-102

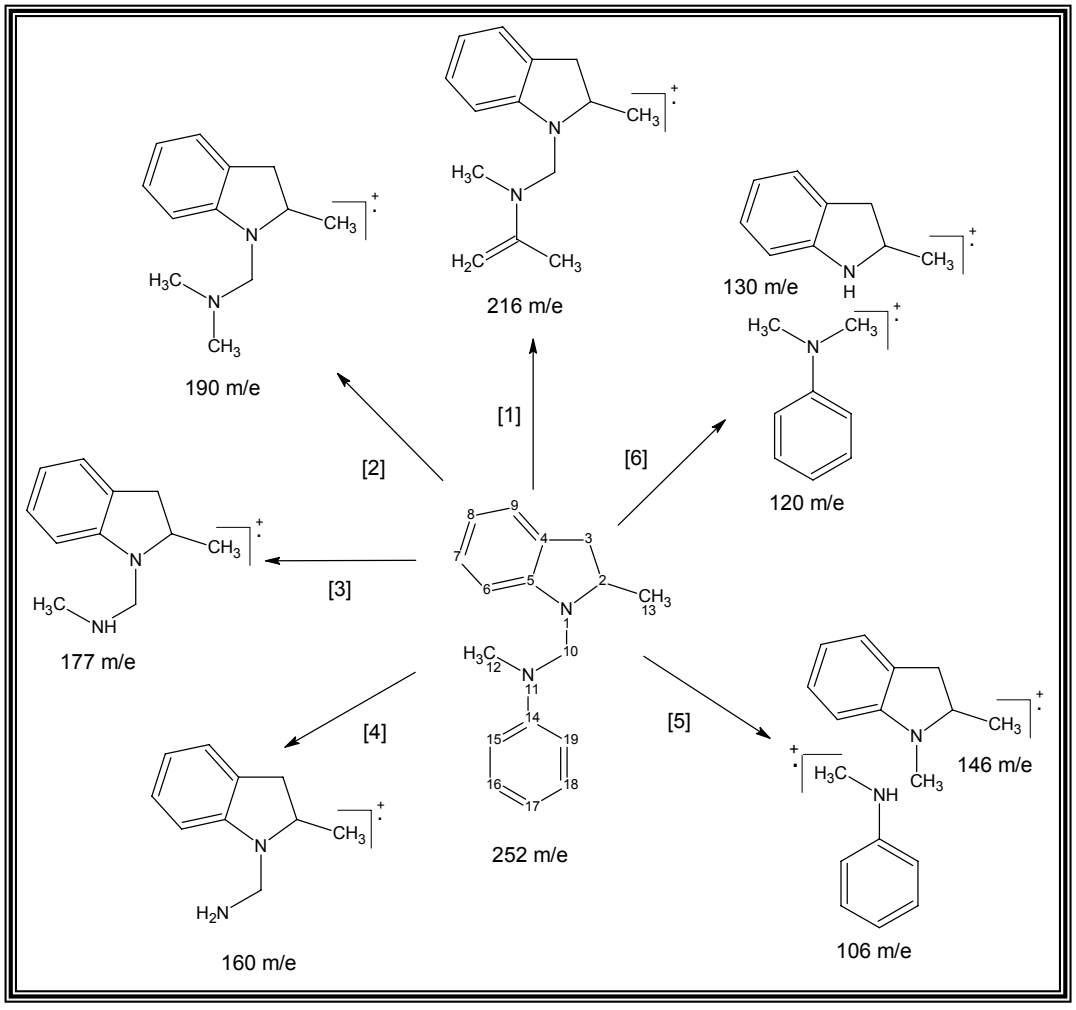


_N_ – Methyl – _N_ - [(2 – methyl - 2, 3 – dihydro - 1_H_ – indol – 1 - yl) methyl] aniline (DNJ-206)

1. The target compound showed characteristic molecular ion peak.
2. Cleavage of the bonds between C_{15}-C_{16} and C_{18}-C_{19} gave characteristic peak at 216 m/e. [1]
3. Cleavage of the bonds between C_{14}-C_{15} and C_{14}-C_{19} gave characteristic peak 190 m/e. [2]

4. N_{11}-C_{14} bond cleavage gave characteristic peak at 177 m/e. [3]
5. Cleavage of the bonds between N_{11}-C_{12} and N_{11}-C_{14} gave characteristic peak at 160 m/e. [4]
6. N_{11}-C_{10} bond cleavage gave two characteristic peaks. One peak at 146 m/e which is the BASE PEAK and second peak at 106 m/e. [5]
7. N_1-C_{10} bond cleavage gave two characteristic peaks. One peak at 130 m/e and second peak at 120 m/e which is the second intense peak in the spectrum. [6]

1.6.1.2 FRAGMENTATION PATTERN FOR DNJ-206



1.6.2 IR SPECTRAL STUDY

IR spectra of the synthesized compounds were recorded on **Shimadzu FT-IR 8400** spectrophotometer using Diffused Reflectance Attachment (DRA) system using Potassium Bromide.

In case of DNJ-101 to DNJ-111, there is no characteristic peak obtained in the spectra except C-H stretching and bending and ring skeleton due to the absence of the functional group. In case of DNJ-103 secondary amine of piperazine gave stretching frequency in the region of 3310 to 3500 cm^{-1} and bending vibrations in the region of 1550 to 1650 cm^{-1}. Aliphatic C-N vibrations are found near 1220 cm^{-1}. C-O-C ether linkage also showed a characteristic frequency in DNJ-102.

DNJ-201 to DNJ-210 compounds showed N-H stretching vibrations in the region of 3310 to 3500 cm^{-1} DNJ-206 and DNJ-207. Frequency for m di substitution has been found in DNJ-202 to DNJ-205. DNJ-202 and DNJ-203 showed C-X stretching frequency. C-O-C ether linkage also showed a characteristic frequency in DNJ-205 and DNJ-210.

1.6.3 ^{1}H & ^{13}C NMR SPECTRAL STUDY

^{1}H & ^{13}C NMR (DEPT 135) spectra of the synthesized compounds were recorded on **Bruker Avance II 400 spectrometer**. Making a solution of samples in $CDCl_3$ solvent using tetramethylsilane (TMS) as the internal standard unless otherwise mentioned. Numbers of protons and carbons identified from NMR spectrum and their chemical shift (δ ppm) were in the agreement of the structure of the molecule. *J* values were calculated to identify o, m and p coupling. In some cases, aromatic protons were obtained as multiplet. ^{1}H & ^{13}C NMR (DEPT 135) spectral interpretation can be discussed as under.

^{1}H NMR spectral interpretation of 2-methyl-1-(morpholin-4-ylmethyl) indoline (DNJ-102)

1. Three most shielded protons of methyl group (C_{17}) gave multiplet at 1.28 δ ppm. Usually these protons should show their multiplicity as doublet due to the presence of single proton at C_9, but two nitrogen atoms are present in the molecule and thus due to their effect these methyl protons coupled with one proton of methine group (C_9) and another two protons of methylene group (C_{10}) and gave multiplet.
2. Four protons of two morpholinyl methylene groups attached with the nitrogen atom gave triplet at 2.45 δ ppm, while another four protons of rest of the two morpholinyl methylene groups attached with the oxygen atom gave triplet at 3.73 δ ppm.
3. Two protons of C_{10} carbon atom splitted into two which showed singlet for each proton at 2.62 δ ppm and at 2.83 δ ppm respectively.
4. One proton of methine group (C_9) gave quartet at 3.14 δ ppm which actually should show multiplet due to the presence of three protons of methyl group (C_{17}) and two protons of methylene group (C_{10}).
5. Two protons of methylene group (C_7) became deshielded due to the two nitrogen atoms and gave singlet at 4.44 δ ppm.
6. Two aromatic protons of C_{13} and C_{15} methine groups gave multiplet at 6.63 δ ppm while another two aromatic protons of C_{14} and C_{16} methine groups gave multiplet at 7.00 δ ppm.

^{13}C NMR spectral interpretation of 2-methyl-1-(morpholin-4-ylmethyl) indoline (DNJ-102)

1. Methyl group-C_{17} carbon gave peak at 14.35 δ ppm chemical shift.
2. 37.55 δ ppm chemical shift is due to the methylene group C_{10} carbon.
3. Two methylene groups of morpholine ring, C_3 and C_5 carbons gave peak at 51.48 δ ppm chemical shift.
4. C_9 carbon attached to the C_{17} methyl group and nitrogen atom of the indoline ring showed chemical shift in downfield at 59.46 δ ppm due to the adjacent nitrogen atom.

5. Another two methylene groups of morpholine ring C_2 and C_6 showed chemical shift in the downfield at 67.05 δ ppm which is due to the oxygen atom directly attached to both the methylene groups.
6. Bridged methylene C_7 carbon gave peak in downfield at 71.45 δ ppm comparatively to other methylene groups due to the two nitrogen atoms of morpholine ring and indoline ring.
7. 77.86, 77.54 and 77.22 δ ppm are the characteristic peaks due to the solvent $CDCl_3$.
8. 107.13, 118.06, 124.22, 127.47, 152.47 and 170.84 δ ppm chemical shifts are due to the aromatic carbon atoms of phenyl ring C_{13}, C_{15}, C_{16}, C_{14}, C_{11} and C_{12} respectively.

Peaks obtained in the DEPT-135 were in the agreement of the carbons present in the molecule.

^{1}H NMR spectral interpretation of 2-methyl-1-(morpholin-4-ylmethyl) indoline (DNJ-206)

1. Three most shielded protons of methyl group (C_{13}) gave multiplet at 1.32 δ ppm. As discussed earlier, these protons should show their multiplicity as doublet due to the presence of single proton at C_2, but two nitrogen atoms are present in the molecule and thus due to their effect these methyl protons coupled with one proton of methane group (C_2) and another two protons of methylene group (C_3) and gave multiplet.
2. Two protons of methylene groups (C_3) gave quartet at 2.59 δ ppm which actually should give double doublet but it merged to give quartet.
3. Three protons of methyl group (C_{12}) attached to the nitrogen atom gave singlet at 2.85 δ ppm.
4. One proton of methine group (C_2) gave quintet at 4.43 δ ppm which actually should show multiplet due to the presence of three protons of methyl group (C_{13}) and two protons of methylene group (C_3).
5. Two protons of methylene group (C_{10}) became deshielded due to the two nitrogen atoms and gave triplet at 4.76 δ ppm. Actually these two

protons should give singlet at this chemical shift but these two protons will couple with three protons of methyl group (C_{12}) and will show multiplet. In this case, some of the peaks have been merged to each other and thus it showed triplet.

6. Rests of the peaks are due to the aromatic protons, where two aromatic protons of methine groups (C_{15} & C_{19}) gave multiplet at 6.68 δ ppm. Two protons of methine groups (C_6 & C_8) gave multiplet at 6.93 δ ppm while another two aromatic protons (C_9 & C_{17}) gave multiplet at 7.05 δ ppm. Rests of the three protons (C_7, C_{16} & C_{18}) gave multiplet at 7.25 δ ppm. Due to the unusual splitting *J* values could not be calculated.

^{13}C NMR spectral interpretation of 2-methyl-1-(morpholin-4-ylmethyl) indoline (DNJ-206)

1. C_{13} carbon atom became most shielded and showed chemical shift at 19.67 δ ppm.
2. Methylene group of C_3 carbon atom gave peak at 37.58 δ ppm.
3. Methyl group attached to the aromatic nitrogen atom N_{11} showed chemical shift at 38.16 δ ppm.
4. C_2 carbon attached to the methyl group C_{13} gave peak at 59.01 δ ppm.
5. Bridged methylene C_{10} carbon gave peak in down field at 65.80 δ ppm comparatively to other methylene groups due to the two nitrogen atoms of aromatic ring and indoline ring.
6. 107.17, 117.90, 124.12, 126.69, 129.28 and 151.30 δ ppm chemical shifts are due to the aromatic carbon atoms of phenyl ring of indoline C_6, C_8, C_9, C_7, C_4 and C_5 respectively.
7. C_{15} and C_{19} carbons of aromatic ring attached to the nitrogen N_{11} showed chemical shift at 112.53 δ ppm. While another two carbons C_{16} and C_{18} showed chemical shift at 127.52 δ ppm.
8. 116.28 and 149.34 δ ppm chemical shifts are due to the carbon atoms of the aromatic ring attached to the nitrogen atom N_{11} C_{17} and C_{14} respectively.

Peaks obtained in the DEPT-135 were in the agreement of the carbons present in the molecule.

1.6.4 ELEMENTAL ANALYSIS

Elemental analysis of the synthesized compounds was carried out on **Vario EL Carlo Erba 1108** which showed calculated and found percentage values of Carbon, Hydrogen and Nitrogen in support of the structure of synthesized compounds. The spectral and elemental analysis data are given for individual compounds.

1.7 ANALYTICAL DATA

2-METHYL-1-(PIPERIDIN-1-YLMETHYL) INDOLINE (DNJ-101): IR (KBr, cm^{-1}): 3070 (Ar-H), 2960 (–CH_3), 2860 (–CH_2), 1605, 1500, 1483, 1460 (Ar-H), 1368 (C-N, Ar, 3°); MS m/z = 230 (M^+); Anal. Calcd. for $C_{15}H_{22}N_2$: C, 78.21; H, 9.63; N, 12.16. Found: C, 78.19; H, 9.64; N, 12.17.

2-METHYL-1-(MORPHOLIN-4-YLMETHYL) INDOLINE (DNJ-102): IR (KBr, cm^{-1}): 3068 (Ar-H), 2957 (–CH_3), 2852 (–CH_2), 1606, 1486, 1455 (Ar-H), 1365 (C-N, Ar, 3°), 1067 (C-O-C); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.28 (3H, m), 2.45 (4H, t), 2.62 (1H, s), 2.83 (1H, s), 3.14 (1H, qt), 3.73 (4H, t), 4.44 (2H, s), 6.63 (2H, m), 7.00 (2H, m); ^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm) 170.84, 152.47, 127.47, 124.22, 118.06, 107.13, 71.45, 67.05, 59.46, 51.48, 37.55, 14.35; MS m/z = 232 (M^+); Anal. Calcd. for $C_{14}H_{20}N_2O$: C, 72.38; H, 8.68; N, 12.06. Found: C, 72.36; H, 8.64; N, 12.09.

2-METHYL-1-(PIPERAZIN-1-YLMETHYL) INDOLINE (DNJ-103): IR (KBr, cm^{-1}): 3234 (-NH), 3020 (Ar-H), 2966 (–CH_3), 2839 (–CH_2), 1604, 1556, 1538, 1463 (Ar-H), 1371 (C-N, Ar, 3°), 1314 (C-N, Ar, 2°); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.31 (3H, m), 1.89 (1H, s), 2.58 (8H, m), 2.87 (2H, d), 3.14 (1H, qt), 4.42 (2H, s), 6.59 (2H, m), 6.98 (2H, m); ^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm) 157.37, 133.13, 132.08, 128.86, 122.26, 112.39, 75.54, 63.98, 56.09,

45.48, 41.99, 24.52; MS m/z = 231 (M^+); Anal. Calcd. for $C_{14}H_{21}N_3$: C, 72.69; H, 9.15; N, 18.16. Found: C, 72.71; H, 9.14; N, 18.15.

2-METHYL-1-[(4-METHYL PIPERAZIN-1-YL) METHYL] INDOLINE (DNJ-104): IR (KBr, cm^{-1}): 3065 (Ar-H), 2958 (–CH_3), 2862 (–CH_2), 1562, 1499 1475, 1458 (Ar-H), 1368 (C-N, Ar, 3°); MS m/z = 245 (M^+); Anal. Calcd. for $C_{15}H_{23}N_3$: C, 73.43; H, 9.45; N, 17.13. Found: C, 73.41; H, 9.45; N, 17.15.

1-[(4-ETHYL PIPERAZIN-1-YL) METHYL]-2-METHYL INDOLINE (DNJ-105): IR (KBr, cm^{-1}): 3066 (Ar-H), 2958 (–CH_3), 2860 (–CH_2), 1560, 1499, 1473, 1458 (Ar-H), 1370 (C-N, Ar, 3°); MS m/z = 259 (M^+); Anal. Calcd. for $C_{16}H_{25}N_3$: C, 74.09; H, 9.71; N, 16.20. Found: C, 74.11; H, 9.69; N, 16.20.

2-METHYL-1-(PYRROLIDIN-1-YLMETHYL) INDOLINE (DNJ-106): IR (KBr, cm^{-1}): 3071 (Ar-H), 2956 (–CH_3), 2856 (–CH_2), 1562, 1505, 1475, 1463 (Ar-H), 1365 (C-N, Ar, 3°); MS m/z = 216 (M^+); Anal. Calcd. for $C_{14}H_{20}N_2$: C, 77.73; H, 9.32; N, 12.95. Found: C, 77.75; H, 9.31; N, 12.94.

1-[(4-BENZYLPIPERAZIN-1-YL) METHYL]-2-METHYL INDOLINE (DNJ-107): IR (KBr, cm^{-1}): 3067 (Ar-H), 2962 (–CH_3), 2860 (–CH_2), 1562, 1500, 1470, 1462 (Ar-H), 1366 (C-N, Ar, 3°); MS m/z = 321 (M^+); Anal. Calcd. for $C_{21}H_{27}N_3$: C, 78.46; H, 8.47; N, 13.07. Found: C, 78.44; H, 8.49; N, 13.07.

***N*, *N*-DIMETHYL-1-(2-METHYL-2, 3-DIHYDRO-1*H*-INDOL-1-YL) METHAN AMINE (DNJ-108):** IR (KBr, cm^{-1}): 3064 (Ar-H), 2960 (–CH_3), 2862 (–CH_2), 1562, 1498, 1470, 1458 (Ar-H), 1369 (C-N, Ar, 3°); MS m/z = 190 (M^+); Anal. Calcd. for $C_{12}H_{18}N_2$: C, 75.74; H, 9.53; N, 14.72. Found: C, 75.71; H, 9.55; N, 14.73.

***N*-ETHYL-*N*-[(2-METHYL-2, 3-DIHYDRO-1*H*-INDOL-1-YL) METHYL] ETHAN AMINE (DNJ-109):** IR (KBr, cm^{-1}): 3060 (Ar-H), 2964 (–CH_3), 2862 (–CH_2), 1562, 1501, 1475, 1462 (Ar-H), 1373 (C-N, Ar, 3°); MS m/z = 218 (M^+);

Anal. Calcd. for $C_{14}H_{22}N_2$: C, 77.01; H, 10.16; N, 12.83. Found: C, 77.04; H, 10.15; N, 12.81.

***N*-[(2-METHYL-2, 3-DIHYDRO-1*H*-INDOL-1-YL) METHYL]-*N*-PROPYL PROPAN-1-AMINE (DNJ-110):** IR (KBr, cm^{-1}): 3062 (Ar-H), 2962 ($–CH_3$), 2864 ($–CH_2$), 1562, 1499, 1470, 1458 (Ar-H), 1375 (C-N, Ar, 3°); MS m/z = 246 (M^+); Anal. Calcd. for $C_{16}H_{26}N_2$: C, 77.99; H, 10.64; N, 11.37. Found: C, 78.01; H, 10.64; N, 11.35.

***N*-BUTYL-*N*-[(2-METHYL-2, 3-DIHYDRO-1*H*-INDOL-1-YL) METHYL] BUTAN-1-AMINE (DNJ-111):** IR (KBr, cm^{-1}): 3062 (Ar-H), 2962 ($–CH_3$), 2862 ($–CH_2$), 1560, 1500, 1470, 1462 (Ar-H), 1374 (C-N, Ar, 3°); MS m/z = 274 (M^+); Anal. Calcd. for $C_{18}H_{30}N_2$: C, 78.77; H, 11.02; N, 10.21. Found: C, 78.80; H, 11.05; N, 10.19.

***N*-[(2-METHYL-2, 3-DIHYDRO-1*H*-INDOL-1-YL) METHYL] ANILINE (DNJ-201):** IR (KBr, cm^{-1}): 3256 (-NH), 3049 (Ar-H), 2914 ($–CH_3$), 2818 ($–CH_2$), 1558, 1495 1470, 1458 (Ar-H), 1380 (C-N, Ar, 3°), 1333 (C-N, Ar, 2°); MS m/z = 238 (M^+); Anal. Calcd. for $C_{16}H_{18}N_2$: C, 80.63; H, 7.61; N, 11.75. Found: C, 80.65; H, 7.59; N, 11.75.

3-CHLORO-*N*-[(2-METHYL-2, 3-DIHYDRO-1*H*-INDOL-1-YL) METHYL] ANILINE (DNJ-202): IR (KBr, cm^{-1}): 3318 (-NH), 3047 (Ar-H), 2962 ($–CH_3$), 2864 ($–CH_2$), 1562, 1503, 1470, 1460 (Ar-H), 1377 (C-N, Ar, 3°), 1327 (C-N, Ar, 2°), 898 (1,3-di sub.), 750 (C-Cl); MS m/z = 272 (M^+); Anal. Calcd. for $C_{16}H_{17}ClN_2$: C, 70.45; H, 6.28; N, 10.27. Found: C, 70.49; H, 6.24; N, 10.31.

***N*-[(2-METHYL-2, 3-DIHYDRO-1*H*-INDOL-1-YL) METHYL]-3-(TRIFLUOROMETHYL) ANILINE (DNJ-203):** IR (KBr, cm^{-1}): 3346 (-NH), 3030 (Ar-H), 2965 ($–CH_3$), 2887 ($–CH_2$), 1609, 1508, 1489, 1450 (Ar-H), 1374 (C-N, Ar, 3°), 1329 (C-N, Ar, 2°), 993 (C-F), 855 (1,3-di sub.); ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.29 (3H, m), 3.66 (2H, qt), 4.11 (1H, qt), 4.63 (2H, q), 4.90 (1H, s), 6.60 (1H, t), 6.74 (2H, m), 6.88 (2H, m), 7.07 (1H, m), 7.23 (2H, m); ^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm) 171.34, 150.63, 147.66, 147.04,

146.78, 129.90, 129.79, 129.75, 129.71, 129.64, 129.44, 127.56, 127.44, 127.40, 127.20, 126.71, 124.73, 124.68, 124.56, 124.05, 118.79, 118.35, 117.99, 116.36, 116.26, 115.69, 114.82, 114.78, 114.74, 114.32, 114.28, 109.66, 109.62, 109.49, 109.45, 108.94, 107.43, 107.24, 106.97, 106.77, 60.50, 56.66, 37.51, 14.19; MS m/z = 306 (M^+); Anal. Calcd. for $C_{17}H_{17}F_3N_2$: C, 66.66; H, 5.59; N, 9.15. Found: C, 66.70; H, 5.54; N, 9.19.

3-METHYL-*N*-[(2-METHYL-2, 3-DIHYDRO-1*H*-INDOL-1-YL) METHYL] ANILINE (DNJ-204): IR (KBr, cm^{-1}): 3341 (-NH), 3056 (Ar-H), 2956 ($–CH_3$), 2860 ($–CH_2$), 1558, 1501, 1471, 1462 (Ar-H), 1386 (C-N, Ar, 3°), 1330 (C-N, Ar, 2°), 846 (1,3-di sub.); MS m/z = 252 (M^+); Anal. Calcd. for $C_{17}H_{20}N_2$: C, 80.91; H, 7.99; N, 11.10. Found: C, 80.95; H, 7.97; N, 11.08.

3-METHOXY-*N*-[(2-METHYL-2, 3-DIHYDRO-1*H*-INDOL-1-YL) METHYL] ANILINE (DNJ-205): IR (KBr, cm^{-1}): 3273 (-NH), 3051 (Ar-H), 2964 ($–CH_3$), 2864 ($–CH_2$), 1562, 1500, 1470, 1460 (Ar-H), 1380 (C-N, Ar, 3°), 1337 (C-N, Ar, 2°), 1066 (C-O-C), 885 (1,3-di sub.); MS m/z = 268 (M^+); Anal. Calcd. for $C_{17}H_{20}N_2O$: C, 76.09; H, 7.51; N, 10.44. Found: C, 76.05; H, 7.53; N, 10.48.

***N*-METHYL-*N*-[(2-METHYL-2, 3-DIHYDRO-1*H*-INDOL-1-YL) METHYL] ANILINE (DNJ-206):** IR (KBr, cm^{-1}): 3025 (Ar-H), 2953 ($–CH_3$), 2870 ($–CH_2$), 1600, 1562, 1486, 1452 (Ar-H), 1362 (C-N, Ar, 3°); ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.32 (3H, m), 2.59 (2H, qt), 2.85 (3H, s), 4.43 (1H, q), 4.76 (2H, t), 6.68 (2H, m), 6.93 (2H, m), 7.05 (2H, m), 7.25 (3H, m); ^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm) 151.30, 149.34, 129.28, 127.52, 126.69, 124.12, 117.90, 116.28, 112.53, 107.17, 65.80, 59.01, 38.16, 37.58, 19.67; MS m/z = 252 (M^+); Anal. Calcd. for $C_{17}H_{20}N_2$: C, 80.91; H, 7.99; N, 11.10. Found: C, 80.87; H, 8.01; N, 11.12.

***N*-[(2-METHYL-2, 3-DIHYDRO-1*H*-INDOL-1-YL) METHYL]-*N*-PHENYL ANILINE (DNJ-207):** IR (KBr, cm^{-1}): 3230 (-NH), 3014 (Ar-H), 2988 ($–CH_3$), 2850 ($–CH_2$), 1550, 1502, 1473, 1460 (Ar-H), 1377 (C-N, Ar, 3°); MS m/z = 314 (M^+); Anal. Calcd. for $C_{22}H_{22}N_2$: C, 84.04; H, 7.05; N, 8.91. Found: C, 84.08; H, 7.03; N, 8.89.

***N*-[(2-METHYL-2, 3-DIHYDRO-1*H*-INDOL-1-YL) METHYL] PYRIDIN-3-AMINE (DNJ-208):** IR (KBr, cm^{-1}): 3341 (-NH), 3077 (Ar-H), 2945 ($–CH_3$), 2862 ($–CH_2$), 1560, 1500, 1470, 1462 (Ar-H), 1363 (C-N, Ar, 3°), 1325 (C-N, Ar, 2°); MS m/z = 239 (M^+); Anal. Calcd. for $C_{15}H_{17}N_3$: C, 75.28; H, 7.16; N, 17.56. Found: C, 75.32; H, 7.13; N, 17.55.

***N*-[(2-METHYL-2, 3-DIHYDRO-1*H*-INDOL-1-YL) METHYL] PYRIDIN-4-AMINE (DNJ-209):** 3292 (-NH), 3026 (Ar-H), 2941 ($–CH_3$), 2866 ($–CH_2$), 1558, 1496, 1470, 1460 (Ar-H), 1382 (C-N, Ar, 3°), 1323 (C-N, Ar, 2°); MS m/z = 239 (M^+); Anal. Calcd. for $C_{15}H_{17}N_3$: C, 75.28; H, 7.16; N, 17.56. Found: C, 75.31; H, 7.16; N, 17.53.

(2-FURYL METHYL)[(2-METHYL-2, 3-DIHYDRO-1*H*-INDOL-1-YL) METHYL] AMINE (DNJ-210): IR (KBr, cm^{-1}): 3275 (-NH), 3020 (Ar-H), 2965 ($–CH_3$), 2862 ($–CH_2$), 1560, 1500, 1473, 1460 (Ar-H), 1371 (C-N, Ar, 3°), 1335 (C-N, Ar, 2°), 1071 (C-O-C); MS m/z = 242 (M^+); Anal. Calcd. for $C_{15}H_{18}N_2O$: C, 74.35; H, 7.49; N, 11.56. Found: C, 74.31; H, 7.51; N, 11.59.

1.8 RESULTS AND DISCUSSION

In this chapter, known methods were adopted for the preparation of 2-methyl indole as well as 2-methyl indoline with slight modifications in the previously reported methods. 2-methyl indole was reduced to 2-methyl indoline using trifluoroacetic acid and 1 M BH_3.THF in tetrahydrofuran (Method – A) along with its yield optimization. In this preparation method use of trifluoroacetic acid was essential because the TLC analysis of a sample obtained by mixing 2-methyl indole and 1 M BH_3.THF in tetrahydrofuran under nitrogen atmosphere with cooling and then worked up without addition of trifluoroacetic acid, showed only the presence of unreacted 2-methyl indole. In and another reduction method, indole was reduced using zinc dust and 85% phosphoric acid (Method – B) where % yield was comparatively low as compare to Method – A. Reduction of indole was carried out under nitrogen to prevent the aerial oxidation as indole is a unstable compound. Its aerial oxidation results into dimerization or polymerization.

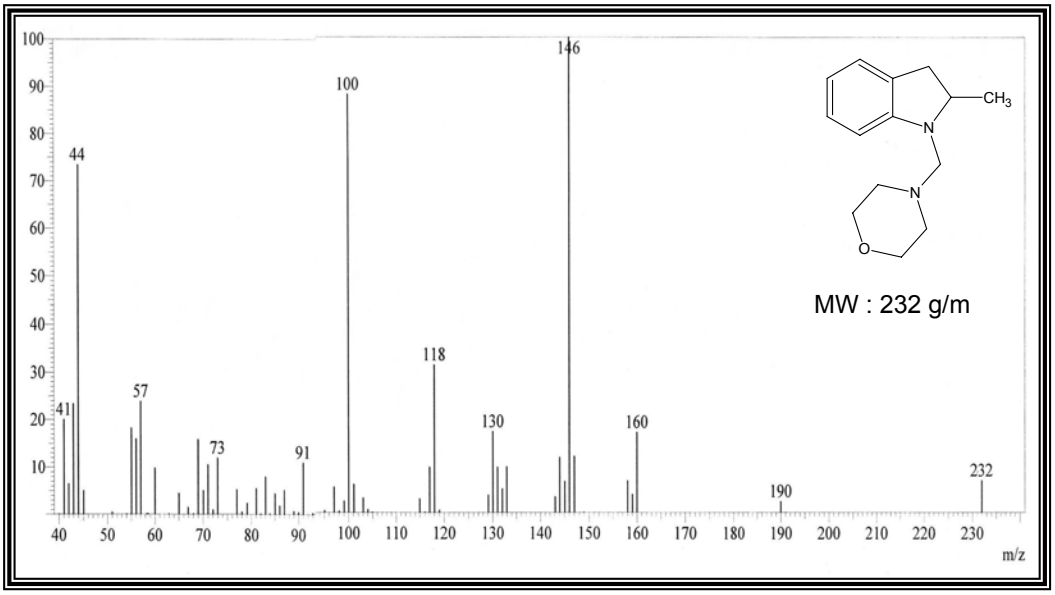
Abonia *et. al.* [a] reported preparation method for the preparation of *N*-Mannich bases of 2-methyl indoline where they used benzotriazole as secondary amine and formaldehyde (37-41% w/w solution). They mixed all the three reagents in diethylether and stirred the reaction mixture at the room temperature and obtained solid product. While the same method was adopted in this chapter also using different liquid secondary amines and primary aromatic amines were used to obtain *N*-Mannich bases and oily liquid type products were obtained in all the cases, but solid product was obtained in case of piperazine. The same was not happened in case of diphenylamine. Thus it may be concluded that the physical state of the product may depend upon the secondary / primary amine used.

1.9 CONCLUSION

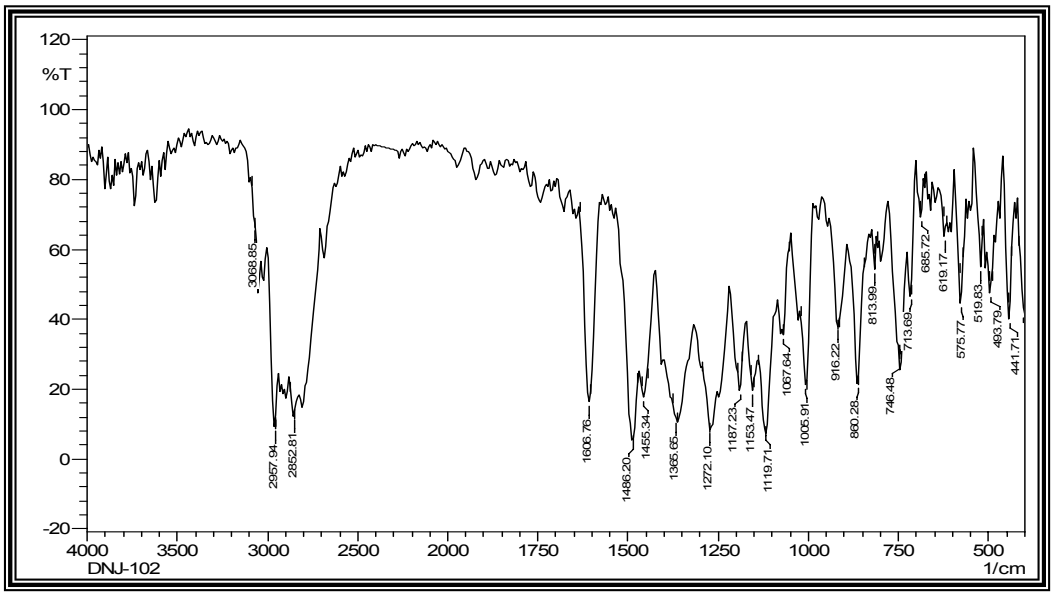
In this chapter, along with yield optimization of the 2-methyl indoline, simple, easy and fast method was adopted first time to prepare new Mannich bases of 2-methyl indoline using formaldehyde solution and primary and secondary amine. DEPT-135 experiment has proved the possible mechanistic path governing this reaction, which is probably hitherto unknown in literature. Antimicrobial data of the synthesized compounds are reported in Chapter – 6.

1.10 SPECTRAL REPRESENTATION OF SYNTHESIZED COMPOUNDS

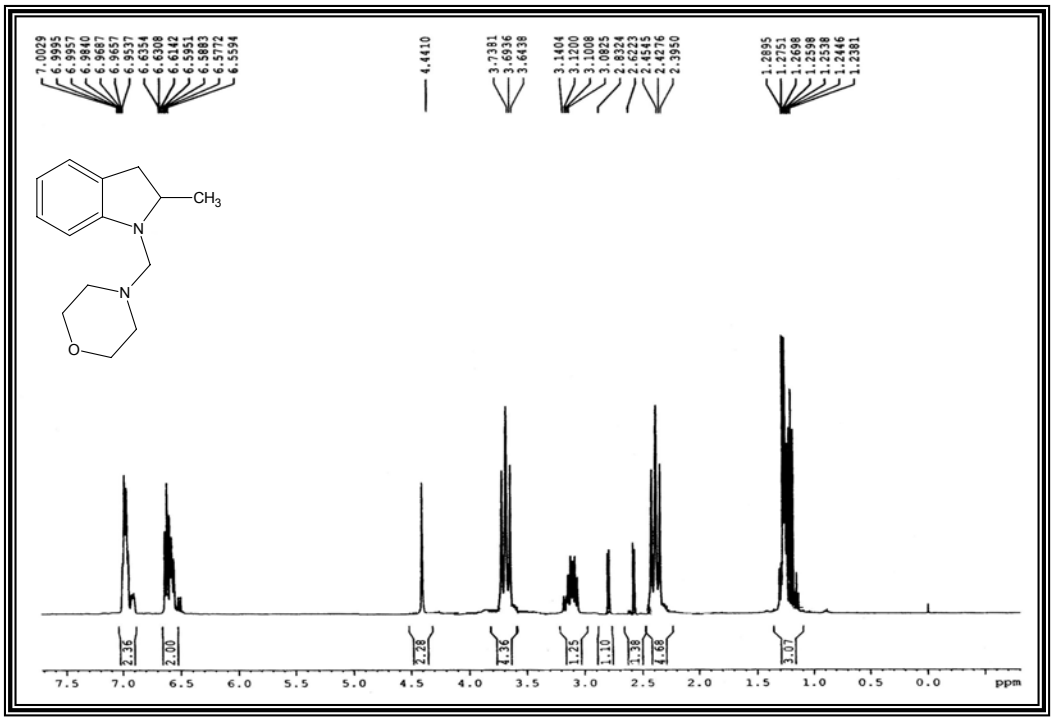
1.10.1 Mass Spectrum of DNJ-102



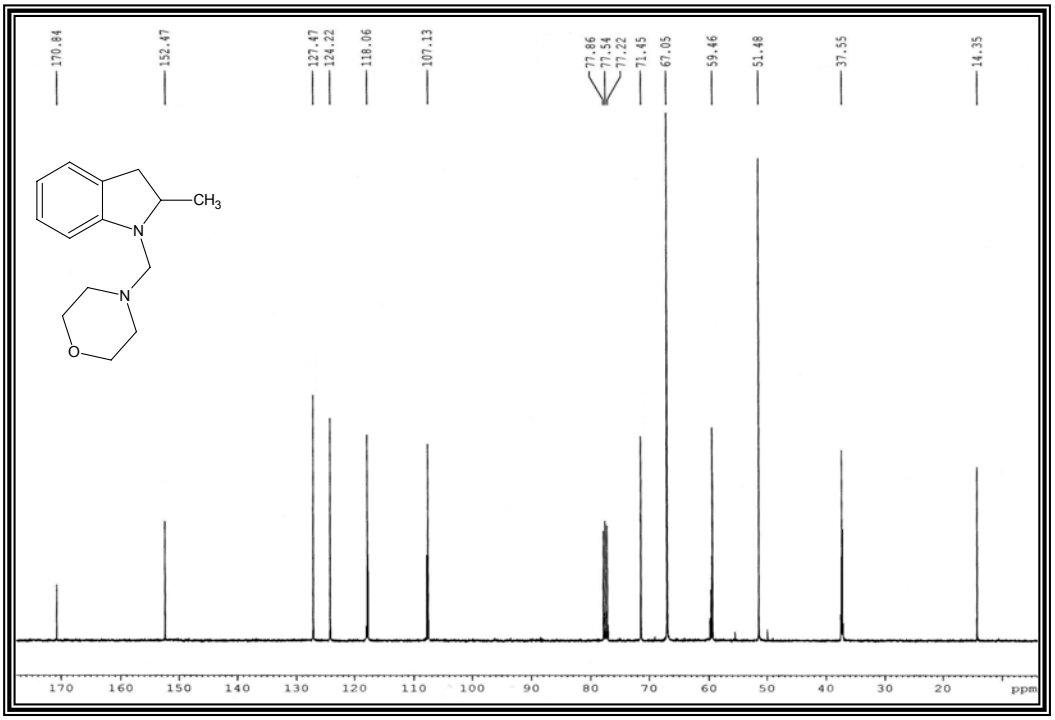
1.10.2 IR Spectrum of DNJ-102

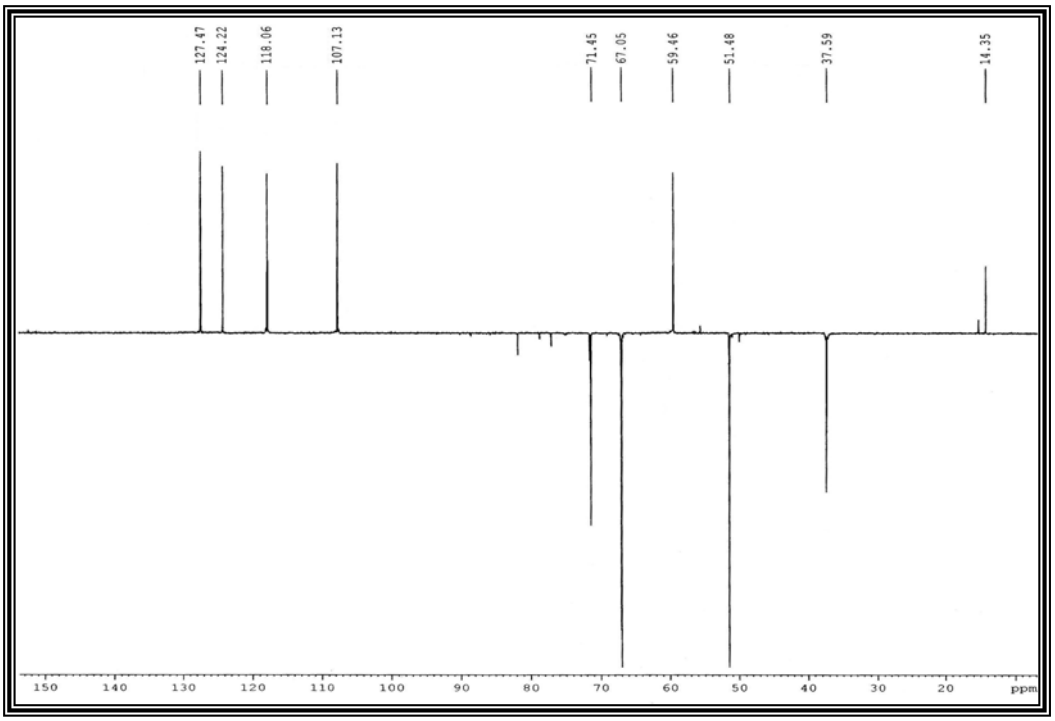


1.10.3 ^{1}H NMR Spectrum of DNJ-102

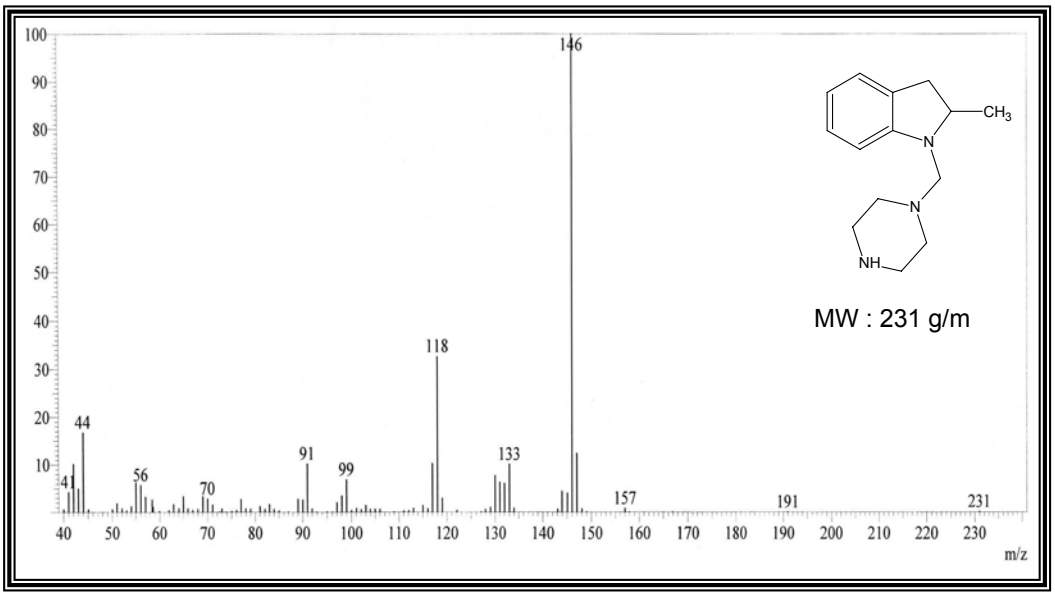


1.10.4 ^{13}C NMR & DEPT 135 Spectrums of DNJ-102

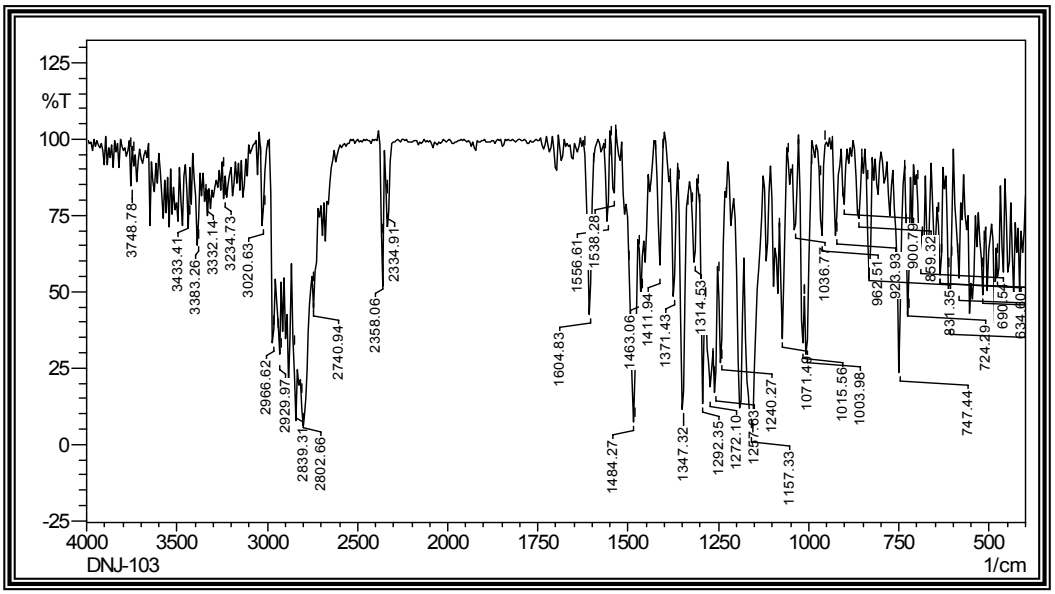




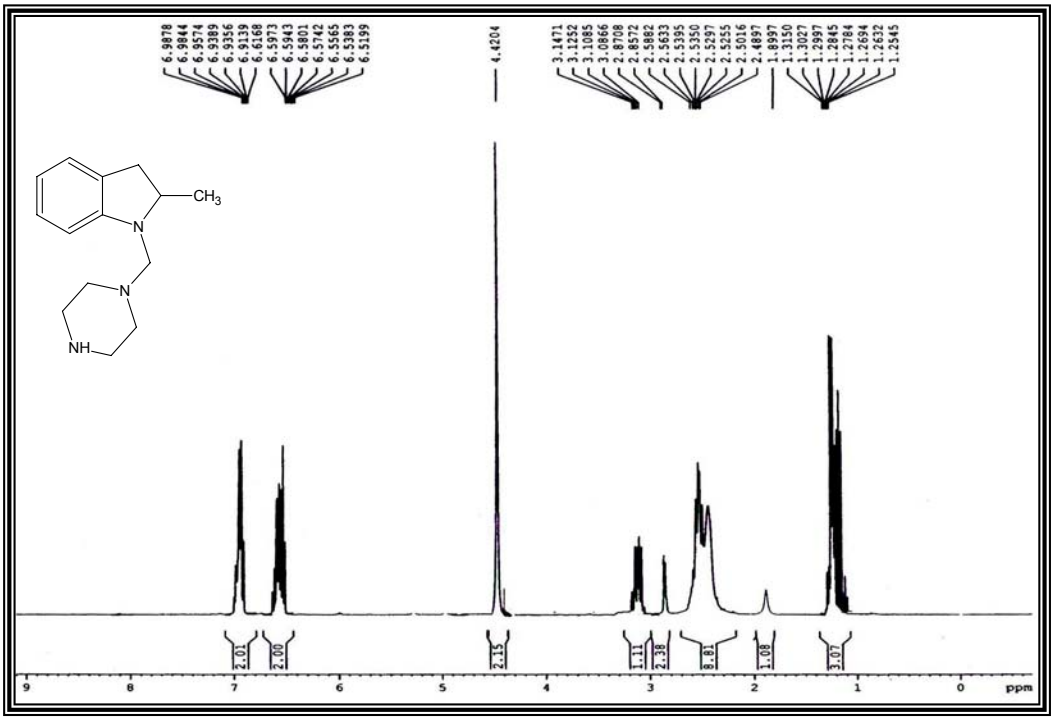
1.10.5 Mass Spectrum of DNJ-103



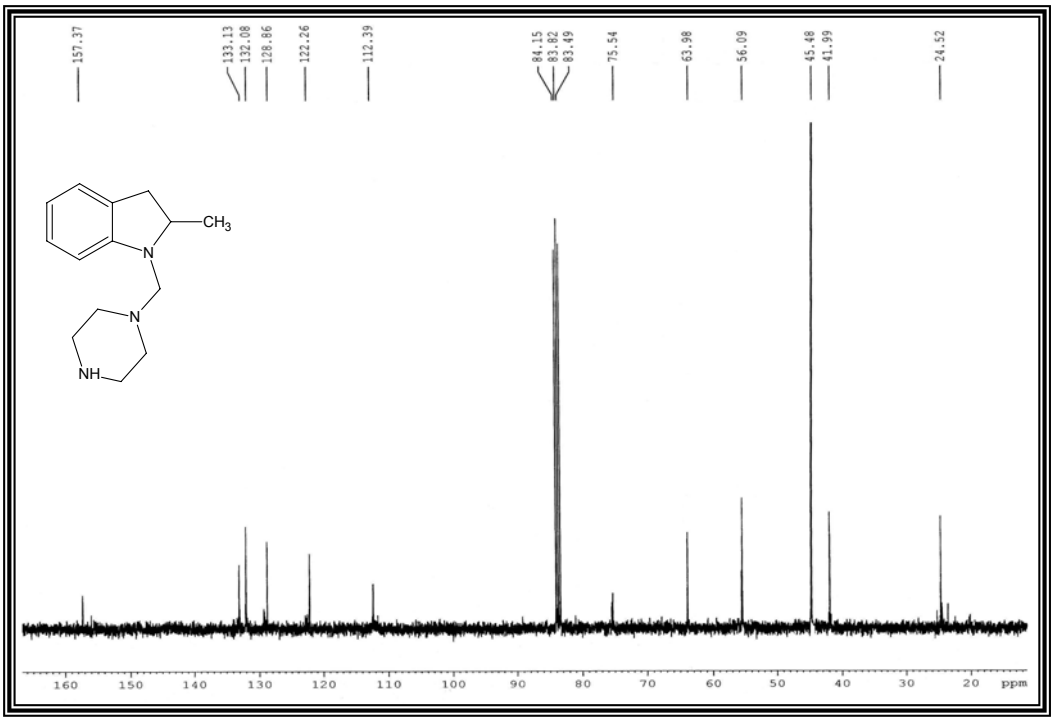
1.10.6 IR Spectrum of DNJ-103

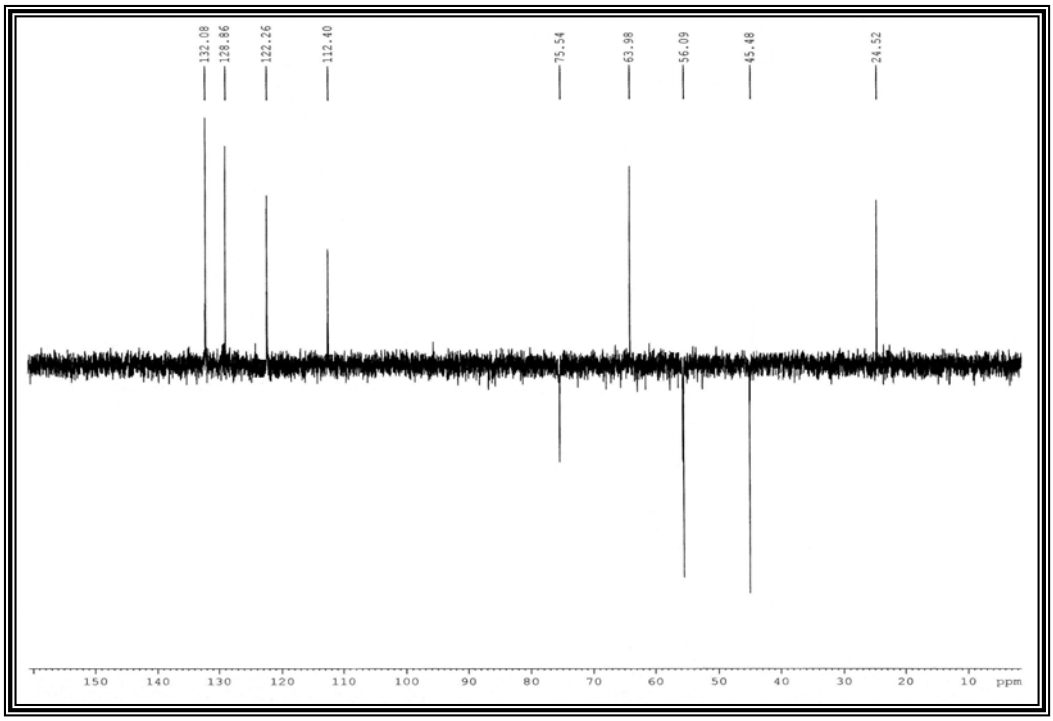


1.10.7 ^{1}H NMR Spectrum of DNJ-103

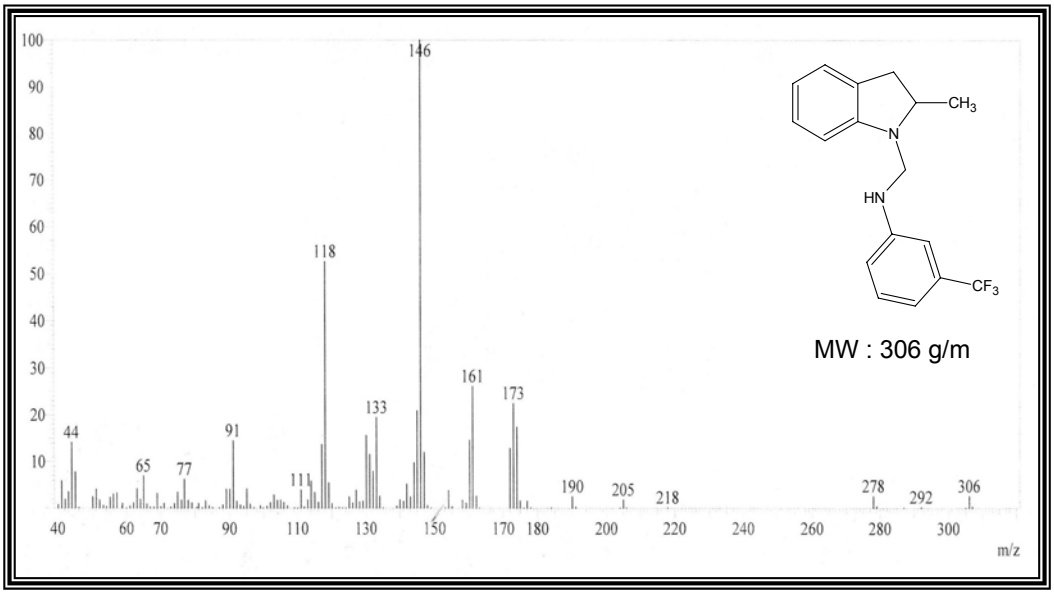


1.10.8 ^{13}C NMR & DEPT 135 Spectrums of DNJ-103

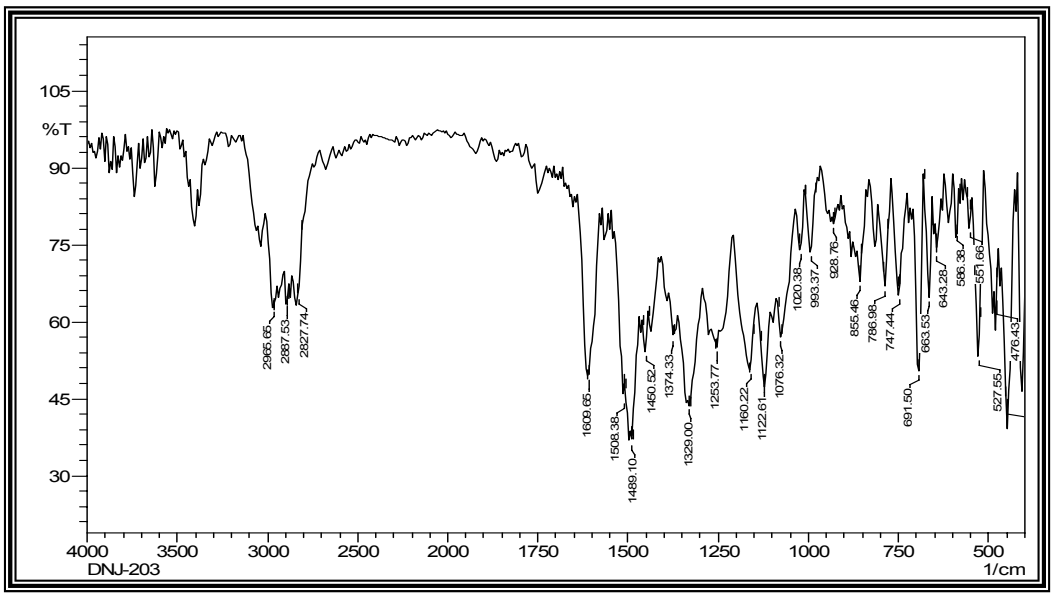




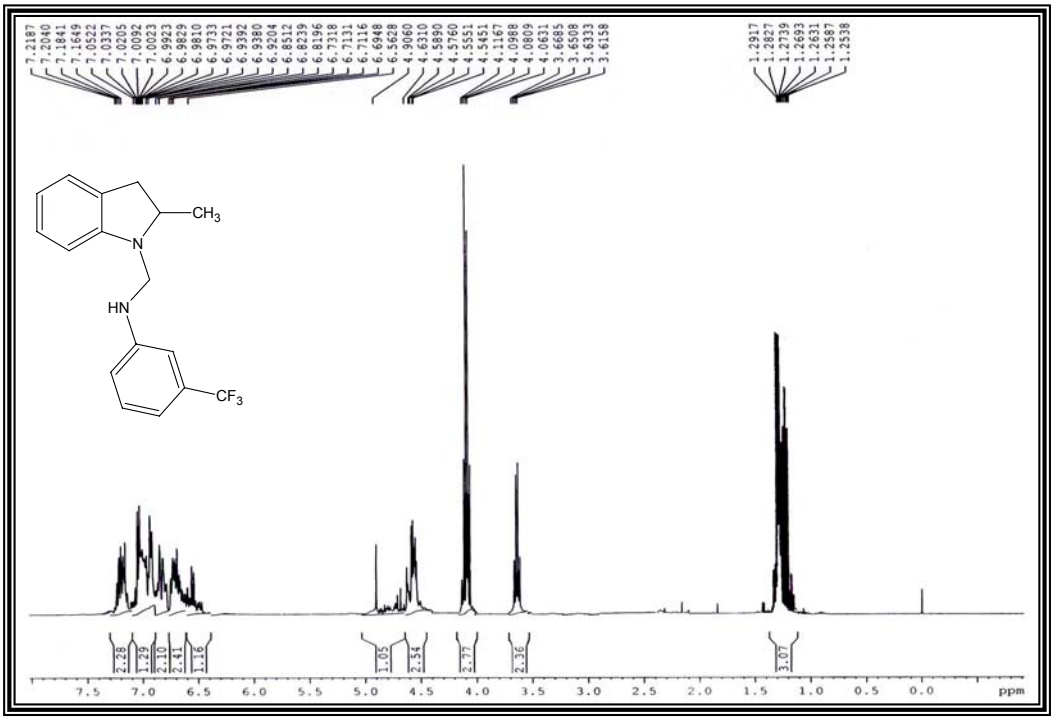
1.10.9 Mass Spectrum of DNJ-203

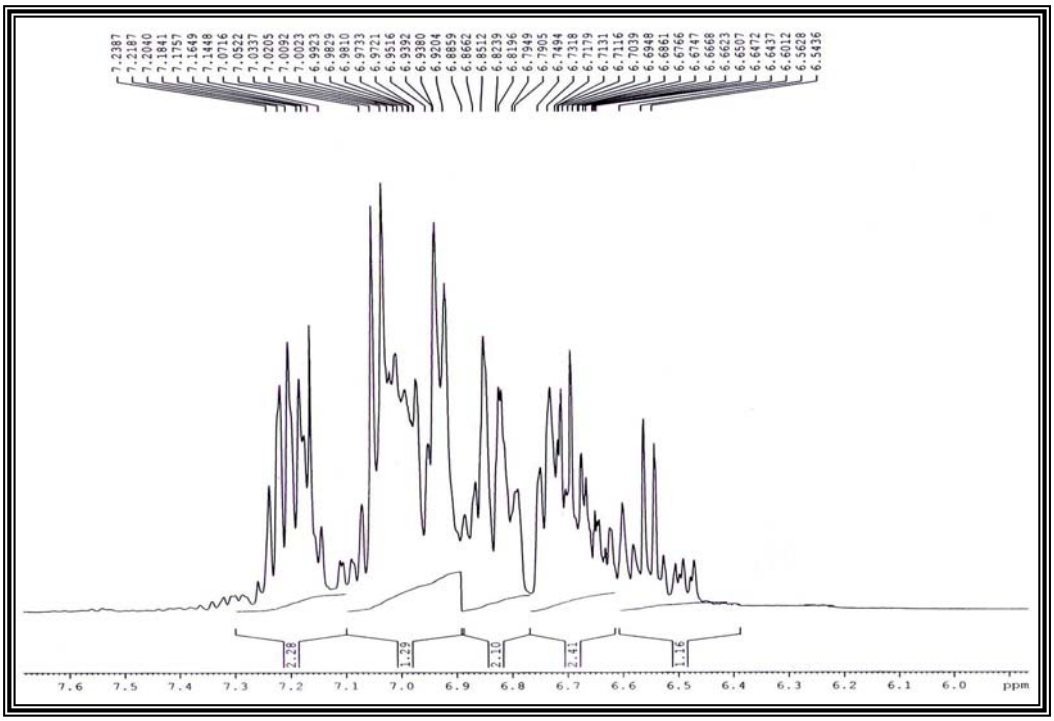


1.10.10 IR Spectrum of DNJ-203

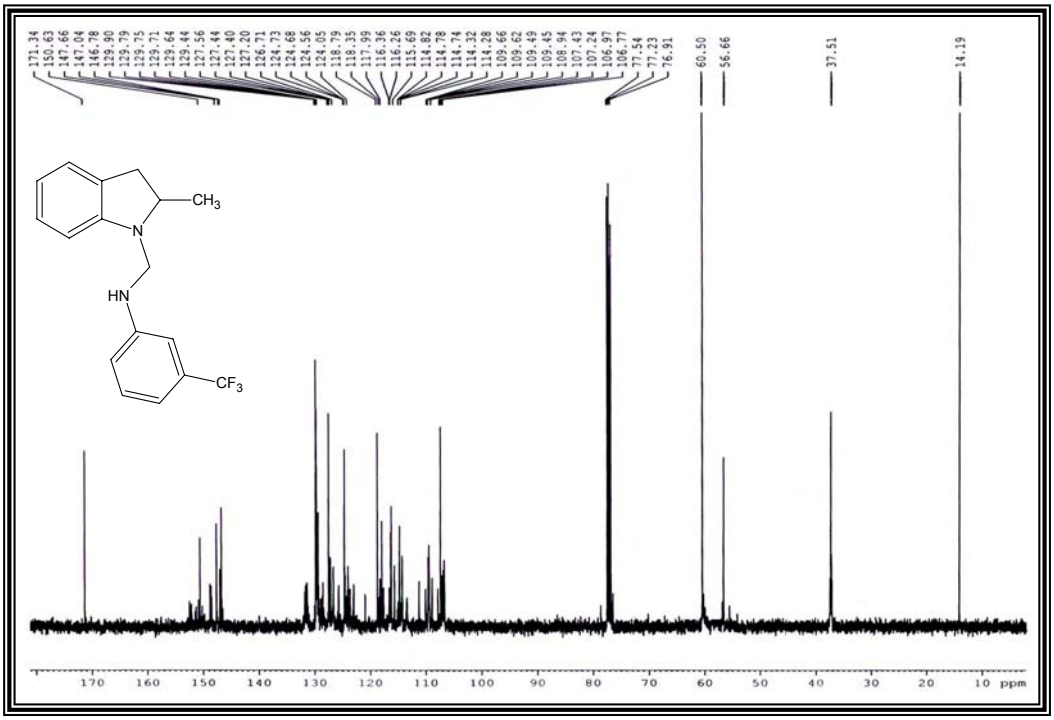


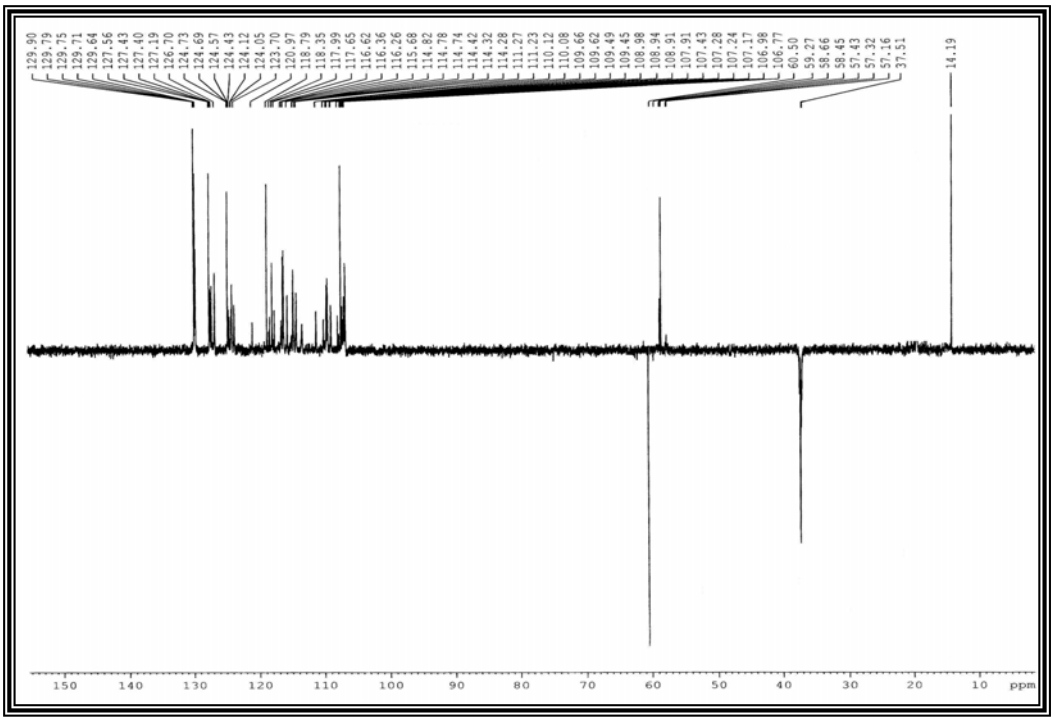
1.10.11 ^{1}H NMR Spectrum of DNJ-203



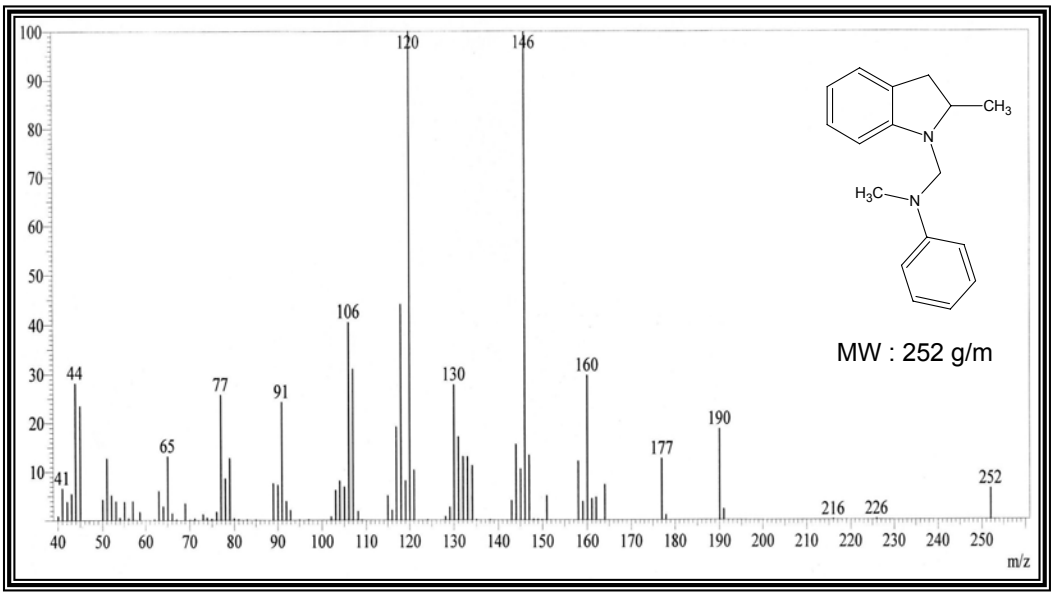


1.10.12 ^{13}C NMR & DEPT 135 Spectrums of DNJ-203

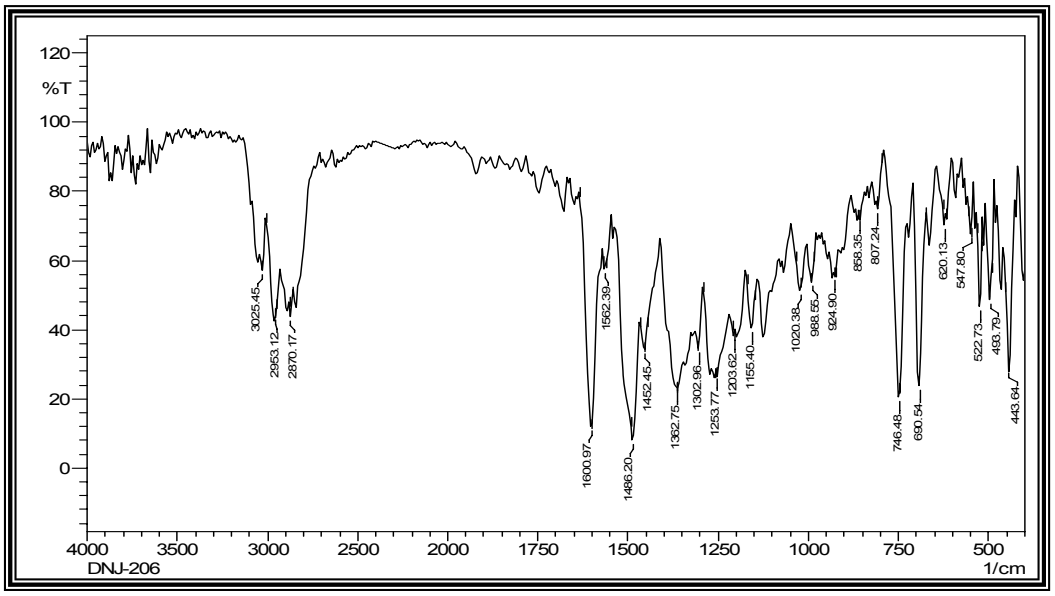




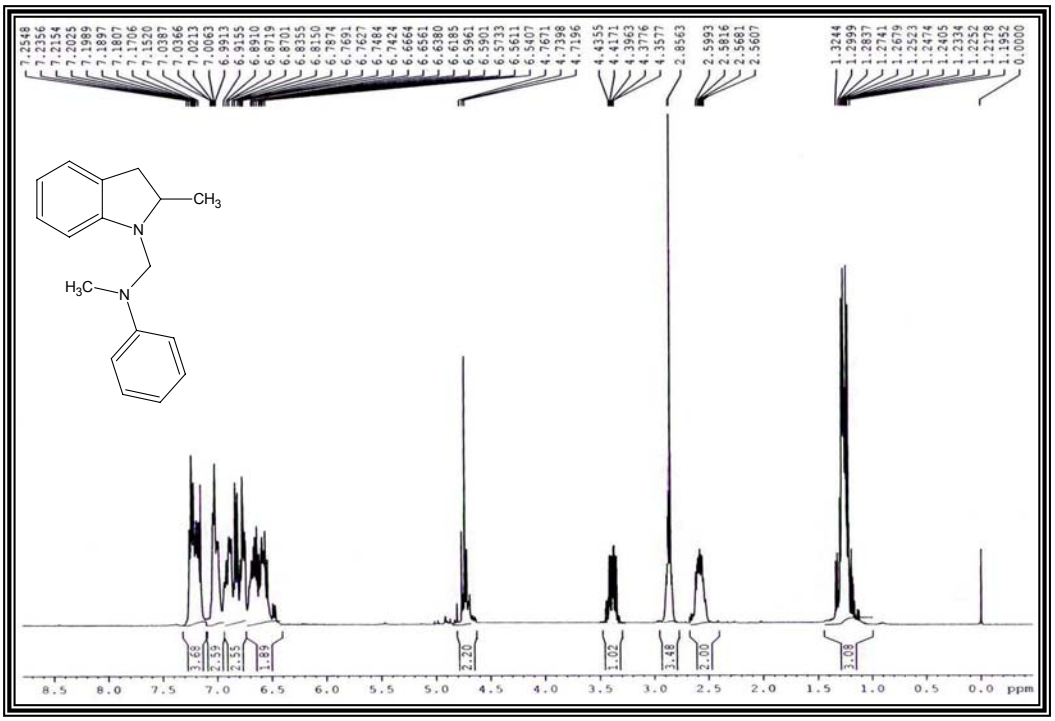
1.10.13 Mass Spectrum of DNJ-206

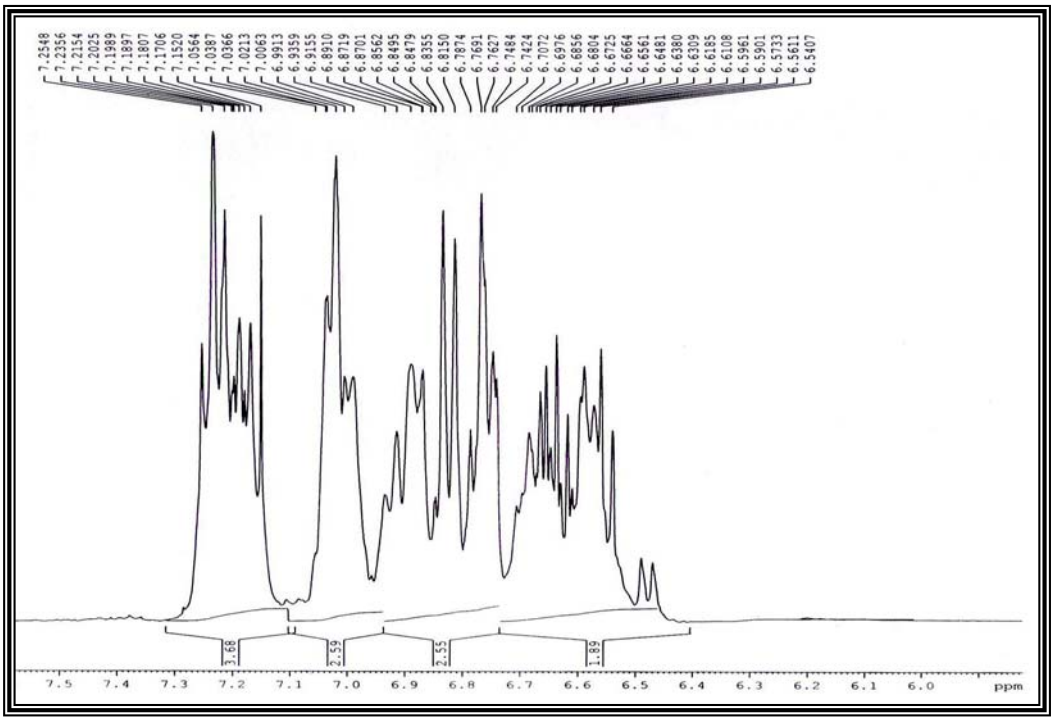


1.10.14 IR Spectrum of DNJ-206

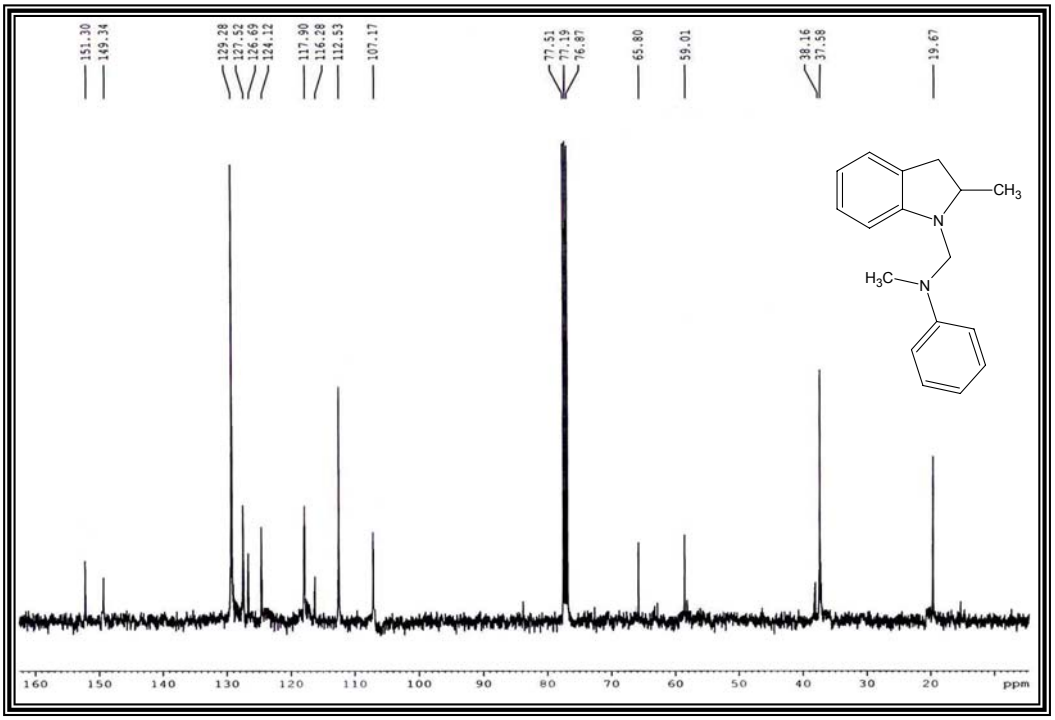


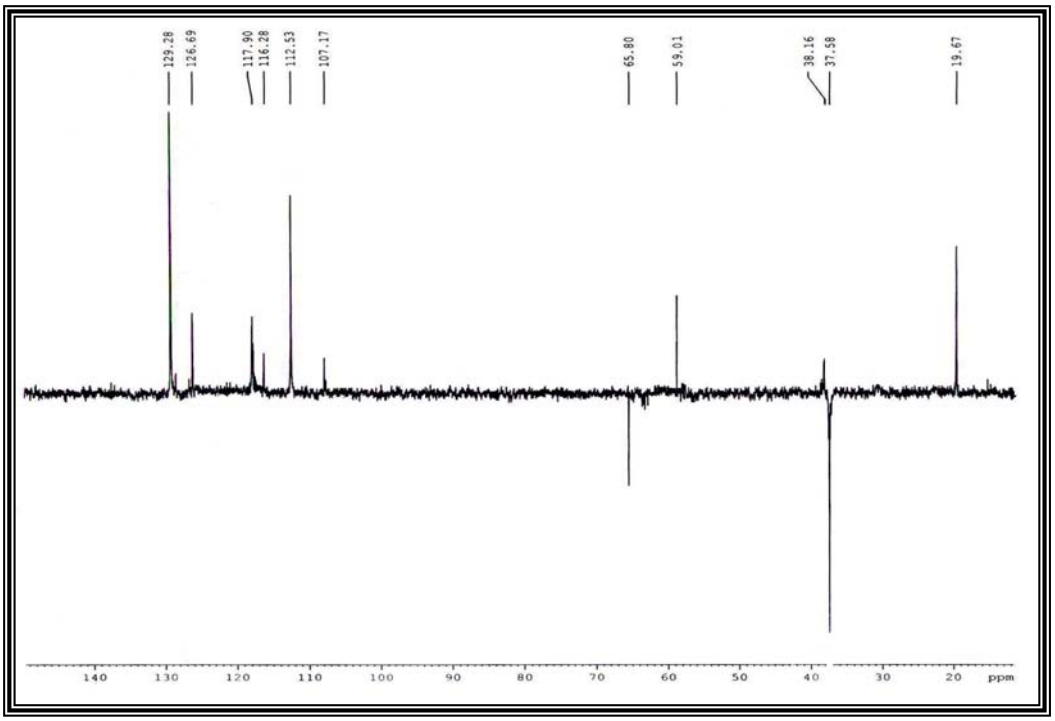
1.10.15 ^{1}H NMR Spectrum of DNJ-206





1.10.16 ^{13}C NMR & DEPT 135 Spectrums of DNJ-206





CHAPTER – 2

MICROWAVE ASSISTED SIMPLE AND FAST *N* – ALKYLATION OF 2-METHYL INDOLINE AND ISATIN MOIETY

2.1 AIM OF CURRENT WORK

Earlier it is already discussed that our group is involved in the synthesis of nitrogen containing heterocycles as they show good biological activity in different classes. Indole nucleus is known to possess different biological activities, thus it was decided to explore the chemistry involving isatin and 2-methyl indoline moiety.

Literature revealed that different types of *N*-alkylation reactions have been carried out on 2-methyl indoline and isatin, which include introduction of acetyl group, introduction of chloroacetyl group and further treatment with secondary amines, preparation of amide linkages and alkylation by means of one and two carbon chains. Looking to the reactivity of *N*-1 position in both the molecules, Mannich reaction had been carried out on 2-methyl indoline in chapter-1 and on isatin in chapter-3. Besides Mannich reaction, 2-methyl indoline and isatin can be suitable moieties for *N*-alkylation reactions. Literature survey revealed that very few *N*-Alkylation reactions have been carried out on 2-methyl indoline as well as on isatin moiety where two to three carbon atom chains have been introduced at *N*-1 position using basic conditions conventionally.

There are many molecules available in the market existing as potent drug *viz.* Chlorpromazine, Fluphenazine, Prochlorperazine, Acetophenazine etc which are having excellent antipsychotic activity. These all molecules contain phenothiazine core moiety as well as long alkyl chain having two to three carbon atoms along with different substituents at the end of the alkyl chain on *N* atom. This inspired us to develop similar type of compounds using different alkyl chains as in above mentioned drugs but on indole nucleus. Thus we used 2-methyl indoline and isatin to develop new molecules. So this was one of the principal rational to carry out the work done in this chapter.

Besides this, green chemistry and especially microwave assisted organic synthesis has drawn a remarkable attention of all chemistry researchers towards its advantages. There are many reactions reported

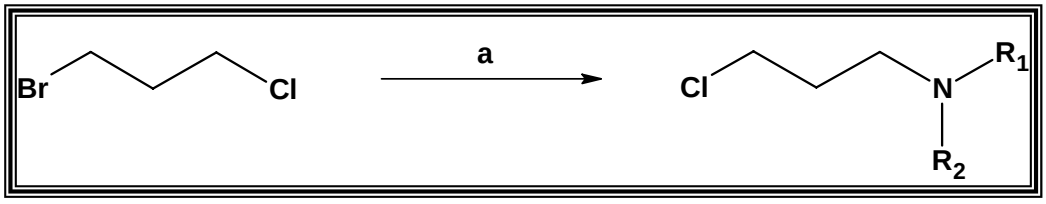
involving microwave assisted organic synthesis. Microwave chemistry is one of the best tools to carry out different reactions on desired molecules with lesser reaction time, lesser energy, easy work up and higher yields with better purity. In this chapter microwave assisted organic synthesis has been adopted to minimize the reaction time as compared to conventional heating and to optimize the results.

Small library of structurally simple but new derivatives of 2-methyl indoline and isatin derivatives was prepared through microwave irradiation, which is reported herein for the first time. In this chapter, few different types of alkyl chains were synthesized by which we could carry out *N*-Alkylation on 2-methyl indoline and isatin. Synthesis and biological evaluation of such important scaffolds was the rational behind the current work done in this chapter.

2.2 REACTION SCHEMES

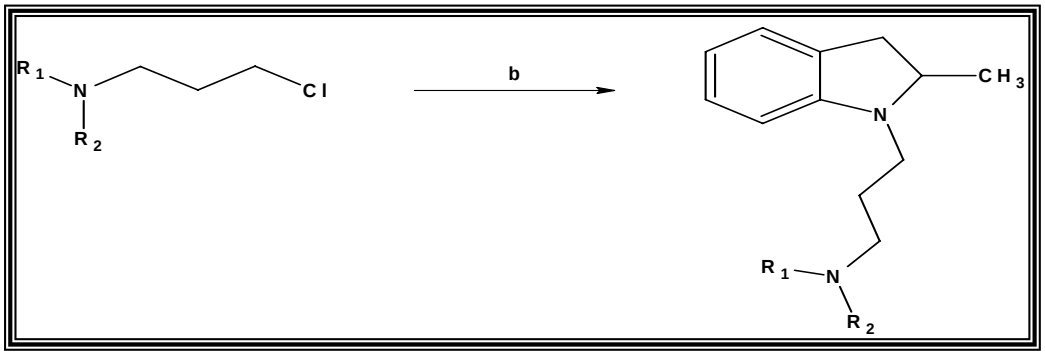
2.2.1 PREPARATION OF 2-METHYL-1-[3-(SUBSTITUTED-1-YL) PROPYL] INDOLINE

STEP – 1



Reagents / Reaction Condition (a): Secondary amines, Toluene/ 75°C, 4 hours.

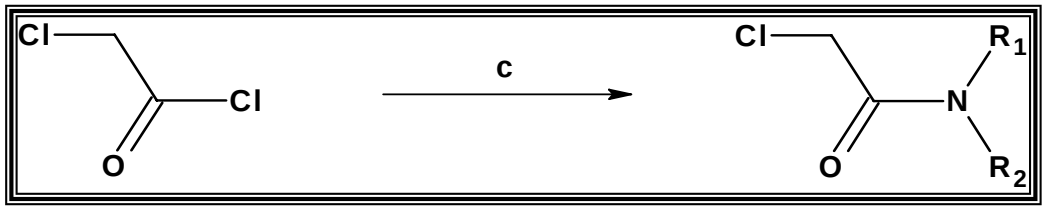
STEP – 2



Reagents / Reaction Condition (b): 2-methyl indoline, K_2CO_3, DMF/ MW, 480 W.

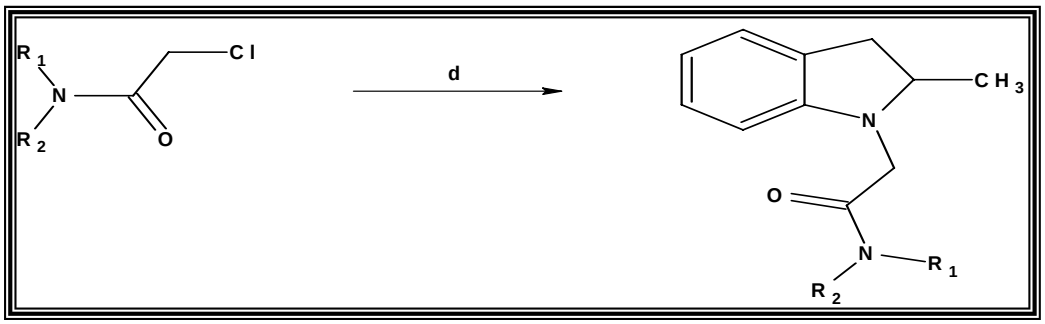
2.2.2 PREPARATION OF 2-METHYL-1-[2-(SUBSTITUTED-1-YL)-2-OXOETHYL] INDOLINE

STEP – 1



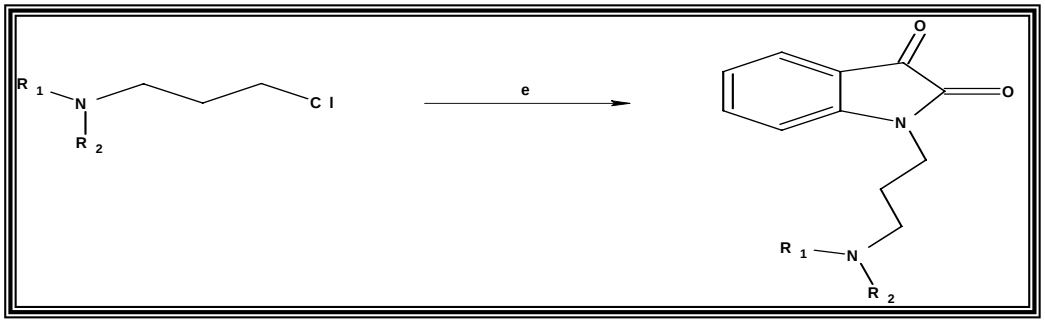
Reagents / Reaction Condition (c): Secondary amines, Benzene, TEA/ 0-5°C, 12 hours, stirring.

STEP – 2



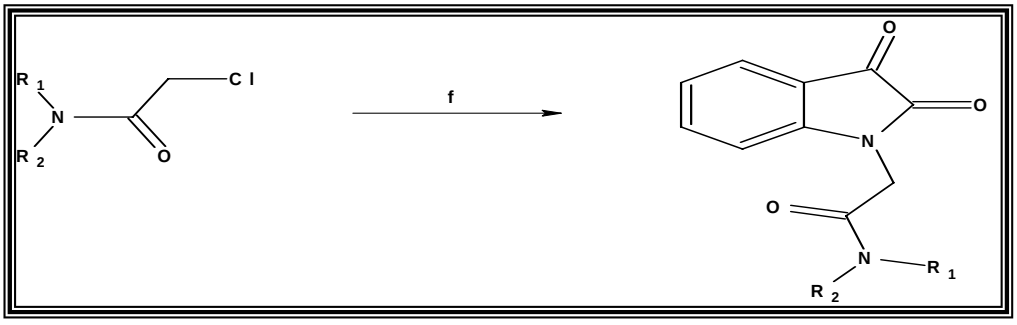
Reagents / Reaction Condition (d): 2-methyl indoline, K_2CO_3, DMF/ MW, 480 W.

2.2.3 PREPARATION OF 1-[3-(SUBSTITUTED-1-YL) PROPYL] - 1*H*-INDOLE-2, 3-DIONE



Reagents / Reaction Condition (e): Isatin, K_2CO_3, DMF/ MW, 480 W.

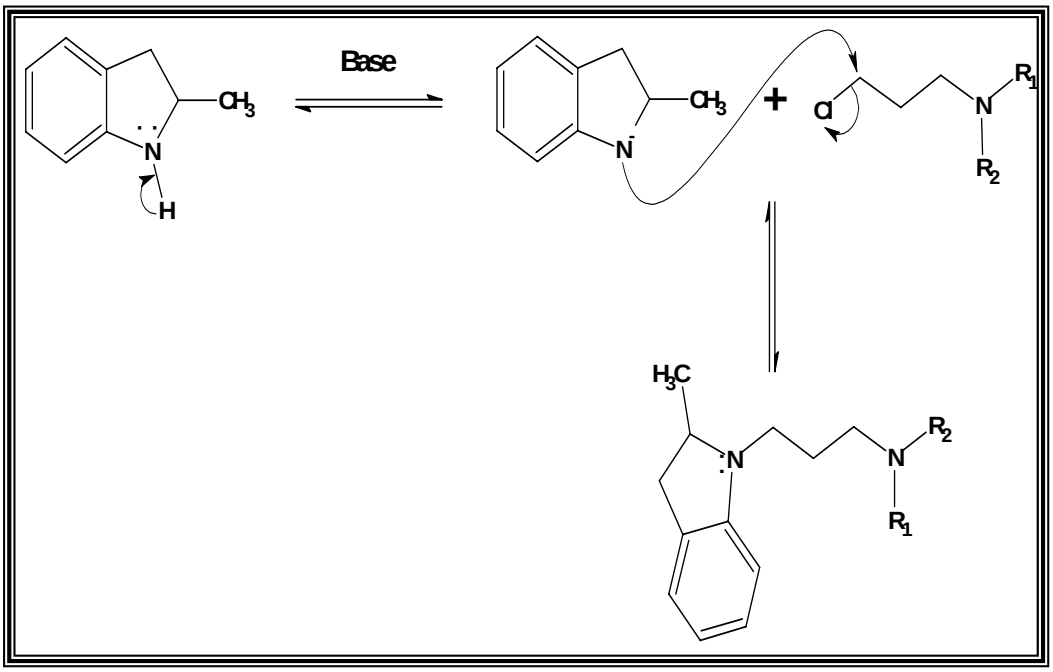
2.2.4 PREPARATION OF 1-(2-SUBSTITUTED-4-YL-2-OXOETHYL)-1*H*-INDOLE-2, 3-DIONE



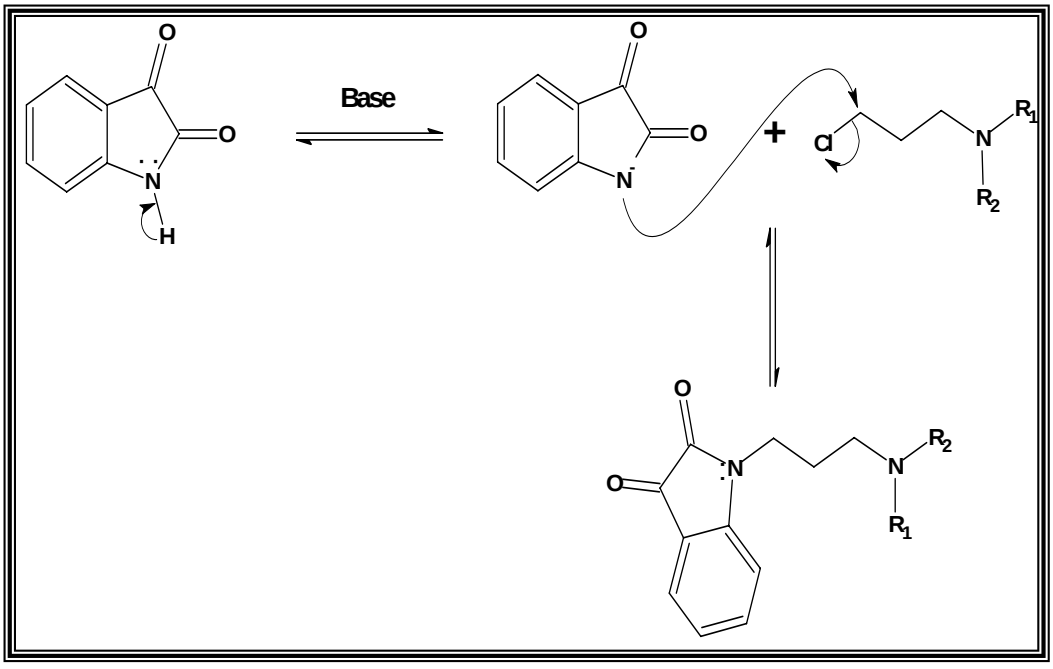
Reagents / Reaction Condition (g): Isatin, K_2CO_3, DMF/ MW, 480 W.

2.3 PLAUSIBLE REACTION MECHANISM

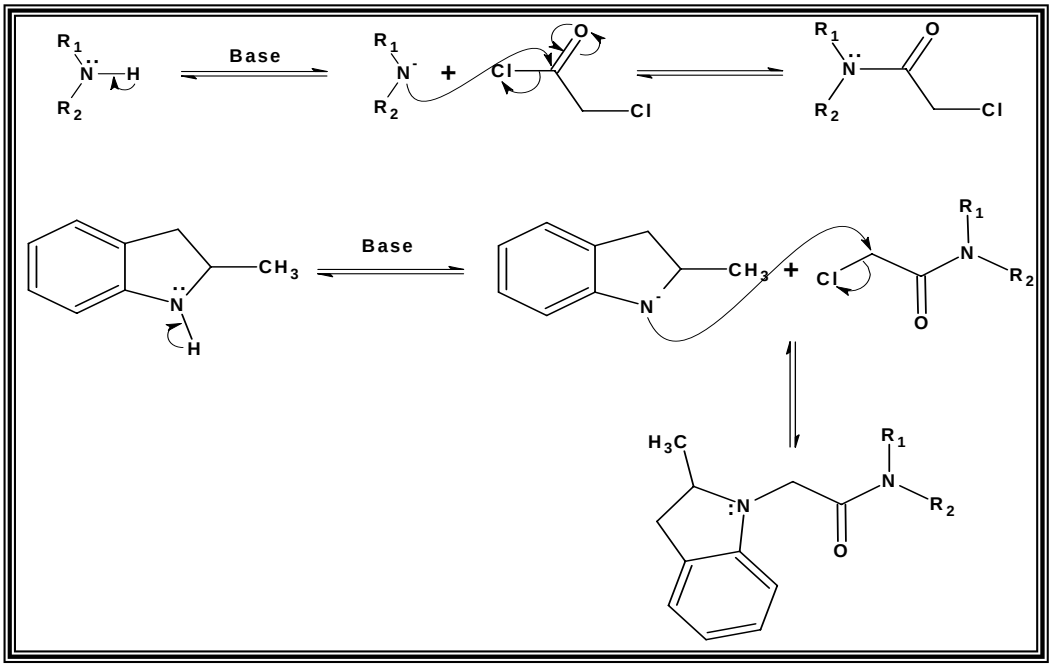
2.3.1 SCHEME - 1



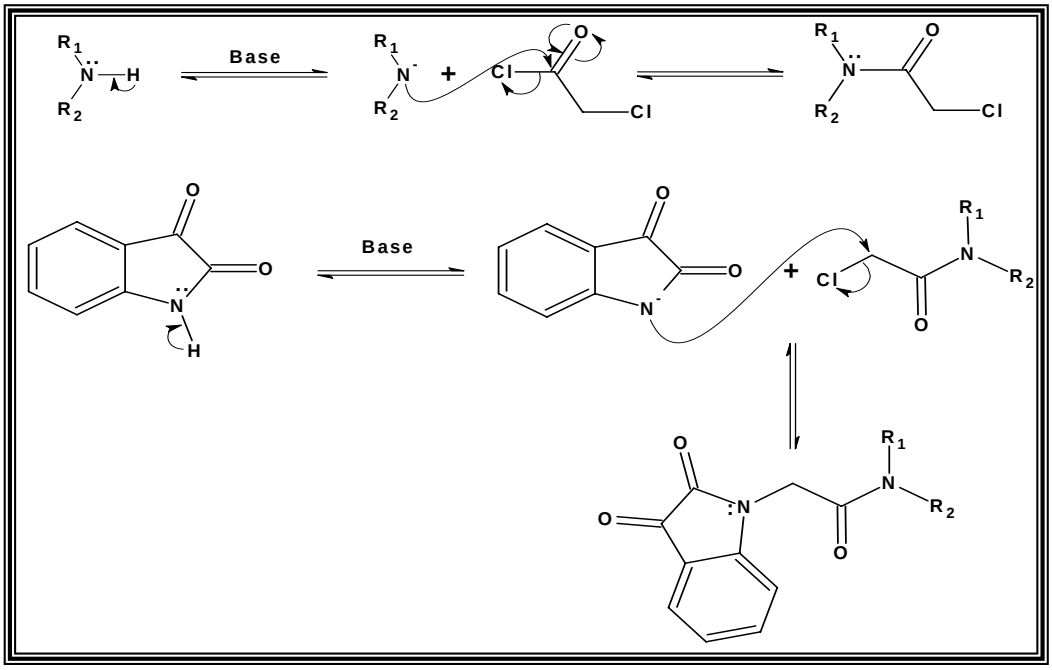
2.3.2 SCHEME - 2



2.3.3 SCHEME - 3



2.3.4 SCHEME - 4



2.4 EXPERIMENTAL

2.4.1 PREPARATION OF 2 – METHYL INDOLINE

It was prepared according to method described in Chapter – 1.

2.4.2 GENERAL PROCEDURE FOR THE PREPARATION OF DNJ-301 TO DNJ-305

STEP – 1 GENERAL PROCEDURE FOR THE PREPARATION OF SIDE CHAINS SC-01 TO SC-05

It was prepared according to the method described by Liu *et. al.* [a] To a stirred solution of 0.127 mole of 1-bromo-3-chloro propane in 100 ml toluene, 0.28 mole of an appropriate secondary amine was added at 75°C. The reaction mixture was stirred at this temperature for 4 hours. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using dichloromethane : methanol (20 : 1) as mobile phase. After the reaction to be completed, it was filtered to remove the precipitation, and the filtrate was concentrated under vacuum to afford crude liquid which was used as such without further purification as the material was pure enough for the subsequent reactions.

Code No.	Substitution R_1 & R_2	% Yield
SC-01	Piperidine	66
SC-02	Morpholine	70
SC-03	1-Methyl Piperazine	62
SC-04	1-Ethyl Piperazine	64
SC-05	1-Benzyl Piperazine	60

STEP – 2 GENERAL PROCEDURE FOR THE PREPARATION OF DNJ-301 TO DNJ-305

[a] H. Liu, L. Smith, Y. Mao, W. Pan, Y.-J. Xu, S. Burdzovic-Wizeman, M. Duneton and W. Wong; *Synth. Commun.*, **2006**, *36 (3)*, 347.

0.01 mole of an appropriate side chain (SC-01 to SC-05) was charged into 250 ml erlenmeyer flask loosely covered with gravity funnel. 0.015 mole of anhydrous potassium carbonate was added followed by addition of 0.01 mole of 2-methyl indoline into above flask. 1 ml of dimethylformamide was added in order to make slurry. The reaction mixture was irradiated under microwave irradiation into household microwave oven (LG MS-192 W) at 480 W for desired time. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using toluene : ethyl acetate (7 : 3) as mobile phase. The reaction mixture was then taken into 50 ml water and it was extracted with ethyl acetate (30 ml X 3), the combined organic layer was washed using water (20 ml X 2). The organic layer was dried on anhydrous sodium sulphate and the solvent was removed under reduced pressure to acquire the product. (Physical data of the synthesized end products are summarized in the table 2.5.1)

Code No.	Substitution R_1 & R_2	Reaction Time (min.) (at 480 W)	% Yield
DNJ-301	Piperidine	6.0	80
DNJ-302	Morpholine	6.3	84
DNJ-303	1-Methyl Piperazine	7.0	79
DNJ-304	1-Ethyl Piperazine	6.5	81
DNJ-305	1-Benzyl Piperazine	7.1	85

2.4.3 GENERAL PROCEDURE FOR THE PREPARATION OF DNJ-401 TO DNJ-405

STEP – 1 GENERAL PROCEDURE FOR THE PREPARATION OF SIDE CHAINS SC-06 TO SC-10

It was prepared according to the method described by Charles and Sharma. [b] A solution of 0.01 mole chloroacetyl chloride in 40 ml dry benzene was added drop wise to a cooled and stirred mixture of 0.01 mole an appropriate secondary amine and 0.01 mole triethylamine in 40 ml dry

[b] E. S. Charles and S. Sharma; *Ind. J. Chem.*, **1987**, *26B(8)*, 752

benzene. The reaction mixture was stirred at room temperature for 12 hours. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using toluene : ethyl acetate (7 : 3) as mobile phase. The reaction mixture was then filtered to remove precipitates of triethylamine hydrochloride. The residue was washed with benzene and the solvent was removed under reduced pressure to give crude product which was used as such without further purification as the material was pure enough for the subsequent reactions.

Code No.	Substitution R_1 & R_2	% Yield
SC-06	Piperidine	56
SC-07	Morpholine	59
SC-08	1-Methyl Piperazine	62
SC-09	1-Ethyl Piperazine	55
SC-10	1-Benzyl Piperazine	50

STEP – 2 GENERAL PROCEDURE FOR THE PREPARATION OF DNJ-401 TO DNJ-405

0.01 mole of an appropriate side chain (SC-06 to SC-10) was charged into 250 ml erlenmeyer flask loosely covered with gravity funnel. 0.015 mole of anhydrous potassium carbonate was added followed by addition of 0.01 mole of 2-methyl indoline into above flask. 1 ml of dimethylformamide was added in order to make slurry. The reaction mixture was irradiated under microwave irradiation into household microwave oven (LG MS-192 W) at 480 W for desired time. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using toluene : ethyl acetate (7 : 3) as mobile phase. The reaction mixture was then taken into 50 ml water and it was extracted with ethyl acetate (30 ml X 3), the combined organic layer was washed using water (20 ml X 2). The organic layer was dried on anhydrous sodium sulphate and the solvent was removed under reduced pressure to acquire the product. (Physical data of the synthesized end products are summarized in the table 2.5.2)

Code No.	Substitution R_1 & R_2	Reaction Time (min.) (at 480 W)	% Yield
DNJ-401	Piperidine	5.5	82
DNJ-402	Morpholine	6.1	80
DNJ-403	1-Methyl Piperazine	6.0	77
DNJ-404	1-Ethyl Piperazine	6.2	79
DNJ-405	1-Benzyl Piperazine	7.0	81

2.4.4 GENERAL PROCEDURE FOR THE PREPARATION OF DNJ-501 TO DNJ-505

0.01 mole of an appropriate side chain (SC-01 to SC-05) was charged into 250 ml erlenmeyer flask loosely covered with gravity funnel. 0.015 mole of anhydrous potassium carbonate was added followed by addition of 0.01 mole of isatin into above flask. 1 ml of dimethylformamide was added in order to make slurry. The reaction mixture was irradiated under microwave irradiation into household microwave oven (LG MS-192 W) at 480 W for desired time. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using toluene : ethyl acetate (7 : 3) as mobile phase. The reaction mixture was then taken into 50 ml water and it was extracted with ethyl acetate (30 ml X 3), the combined organic layer was washed using water (20 ml X 2). The organic layer was dried on anhydrous sodium sulphate and the solvent was removed under reduced pressure to acquire the product. (Physical data of the synthesized end products are summarized in the table 2.5.3)

Code No.	Substitution R_1 & R_2	Reaction Time (min.) (at 480 W)	% Yield
DNJ-501	Piperidine	6.5	81
DNJ-502	Morpholine	7.0	83
DNJ-503	1-Methyl Piperazine	7.4	77
DNJ-504	1-Ethyl Piperazine	7.5	79
DNJ-505	1-Benzyl Piperazine	7.3	82

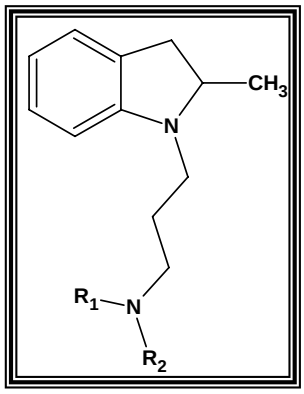
2.4.5 GENERAL PROCEDURE FOR THE PREPARATION OF DNJ-601 to DNJ-605

0.01 mole of an appropriate side chain (SC-06 to SC-10) was charged into 250 ml erlenmeyer flask loosely covered with gravity funnel. 0.015 mole of anhydrous potassium carbonate was added followed by addition of 0.01 mole of isatin into above flask. 1 ml of dimethylformamide was added in order to make slurry. The reaction mixture was irradiated under microwave irradiation into household microwave oven (LG MS-192 W) at 480 W for desired time. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using toluene : ethyl acetate (7 : 3) as mobile phase. The reaction mixture was then taken into 50 ml water and it was extracted with ethyl acetate (30 ml X 3), the combined organic layer was washed using water (20 ml X 2). The organic layer was dried on anhydrous sodium sulphate and the solvent was removed under reduced pressure to acquire the product. (Physical data of the synthesized end products are summarized in the table 2.5.4)

Code No.	Substitution R_1 & R_2	Reaction Time (min.) (at 480 W)	% Yield
DNJ-601	Piperidine	7.5	79
DNJ-602	Morpholine	7.0	81
DNJ-603	1-Methyl Piperazine	7.4	75
DNJ-604	1-Ethyl Piperazine	7.5	80
DNJ-605	1-Benzyl Piperazine	7.3	74

2.5 PHYSICAL DATA TABLES

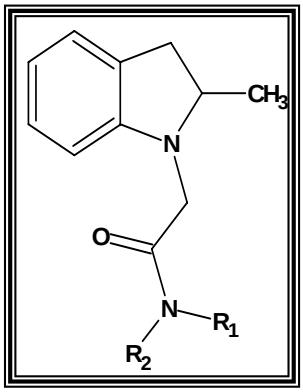
2.5.1 Physical data of 2-methyl-1-[3-(substituted-1-yl) propyl] indoline (DNJ-301 to DNJ-305)



Code	Substitution R_1 & R_2	MF	MW	R_f
DNJ-301	Piperidinyl	$C_{17}H_{26}N_2$	258	0.60
DNJ-302	Morpholinyl	$C_{16}H_{24}N_2O$	260	0.59
DNJ-303	1-methyl piperazinyl	$C_{17}H_{27}N_3$	273	0.62
DNJ-304	1-ethyl piperazinyl	$C_{18}H_{29}N_3$	287	0.64
DNJ-305	1-benzyl piperazinyl	$C_{23}H_{31}N_3$	349	0.70

R_f value was calculated using solvent system = Toluene : Ethyl Acetate (7 : 3)

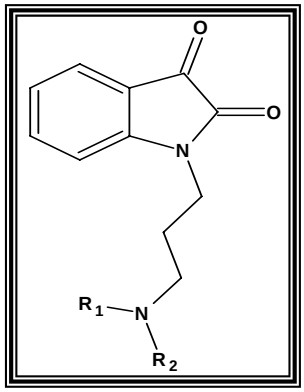
2.5.2 Physical Data of 2-methyl-1-[2-(substituted-1-yl)-2-oxoethyl] indoline (DNJ-401 to DNJ-405)



Code	Substitution R_1 & R_2	MF	MW (g/m)	R_f
DNJ-401	Piperidinyl	$C_{16}H_{22}N_2O$	258	0.46
DNJ-402	Morpholinyl	$C_{15}H_{20}N_2O_2$	260	0.44
DNJ-403	1-methyl piperazinyl	$C_{16}H_{23}N_3O$	273	0.45
DNJ-404	1-ethyl piperazinyl	$C_{17}H_{25}N_3O$	287	0.48
DNJ-405	1-benzyl piperazinyl	$C_{22}H_{27}N_3O$	349	0.53

R_f value was calculated using solvent system = Toluene : Ethyl Acetate (7 : 3)

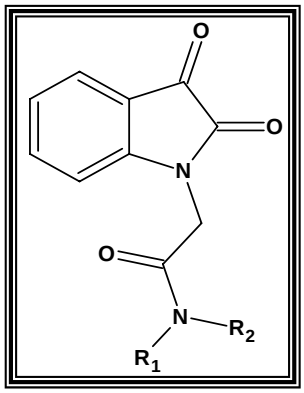
2.5.3 Physical data of 1-[3-(substituted-1-yl) propyl]-1*H*-indole-2, 3-dione (DNJ-501 to DNJ-505)



Code	Substitution R_1 & R_2	MF	MW (g/m)	MP (°C)	R_f
DNJ-501	Piperidinyl	$C_{16}H_{20}N_2O_2$	272	160-162	0.56
DNJ-502	Morpholinyl	$C_{15}H_{18}N_2O_3$	274	168-170	0.55
DNJ-503	1-methyl piperazinyl	$C_{16}H_{21}N_3O_2$	287	178-180	0.57
DNJ-504	1-ethyl piperazinyl	$C_{17}H_{23}N_3O_2$	301	166-168	0.59
DNJ-505	1-benzyl piperazinyl	$C_{22}H_{25}N_3O_2$	363	174-176	0.62

R_f value was calculated using solvent system = Toluene : Ethyl Acetate (7 : 3)

2.5.4 Physical data of 1-(2-substituted-4-yl-2-oxoethyl)-1*H*-indole-2, 3-dione (DNJ-601 to DNJ-605)



Code	Substitution R_1 & R_2	MF	MW (g/m)	MP (°C)	R_f
DNJ-601	Piperidinyl	$C_{15}H_{16}N_2O_3$	272	178-180	0.43
DNJ-602	Morpholinyl	$C_{14}H_{14}N_2O_4$	274	168-170	0.42
DNJ-603	1-methyl piperazinyl	$C_{15}H_{17}N_3O_3$	287	162-164	0.44
DNJ-604	1-ethyl piperazinyl	$C_{16}H_{19}N_3O_3$	301	184-186	0.48
DNJ-605	1-benzyl piperazinyl	$C_{21}H_{21}N_3O_3$	363	174-176	0.43

R_f value was calculated using solvent system = Toluene : Ethyl Acetate (7 : 3)

2.6 SPECTRAL DISCUSSION

2.6.1 MASS SPECTRAL STUDY

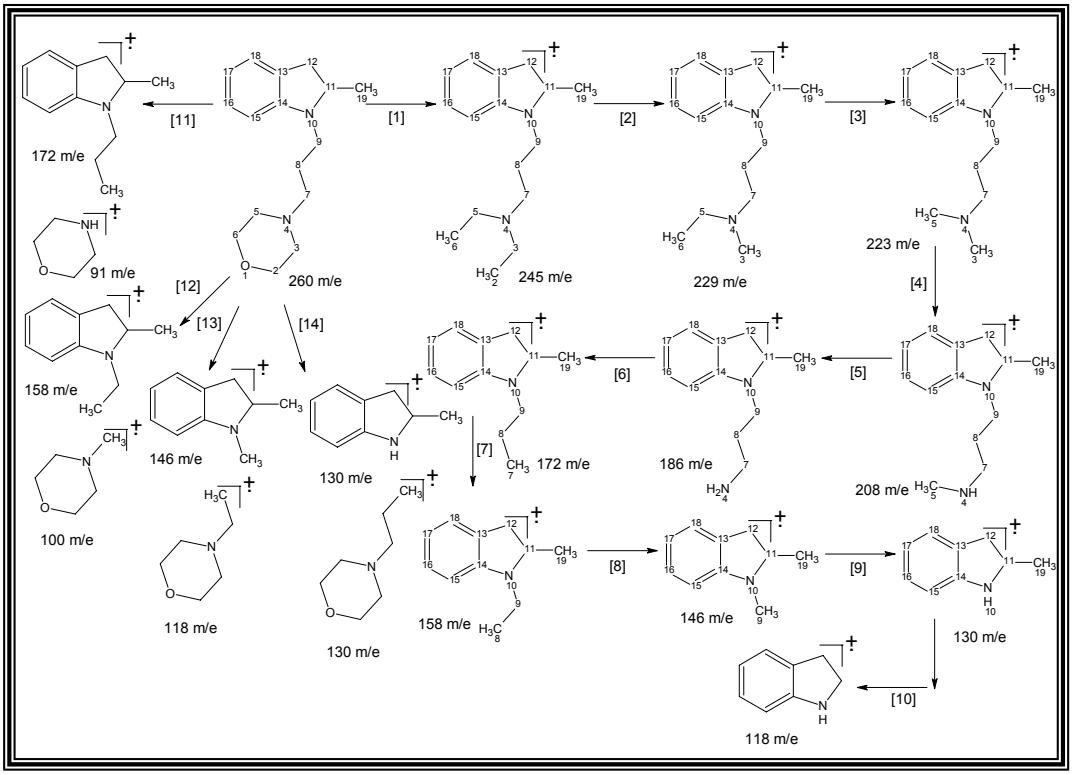
Mass spectra of the synthesized compounds were recorded on **Shimadzu GC-MS QP-2010** model using direct injection probe technique. The molecular ion peak was found in agreement with molecular weight of the respective compound. Characteristic M^{+2} ion peaks with one-third intensity of molecular ion peak were observed in case of compounds having chlorine atom. Fragmentation pattern can be observed to be particular for these compounds and the characteristic peaks obtained for each compound. Probable fragmentation pattern for DNJ-302, DNJ-402, DNJ-502 and DNJ-602 can be discussed as under.

2-methyl-1-(3-morpholin-4-ylpropyl) indoline (DNJ-302)

1. The target compound showed characteristic molecular ion peak.
2. O_1-C_2 and O_1-C_6 bond cleavages gave characteristic peak at 245 m/e. [1]
3. After O_1-C_2 and O_1-C_6 bond cleavages, C_2-C_3 bond cleavage gave characteristic peak at 229 m/e. [2]
4. After C_2-C_3 bond cleavage, C_5-C_6 bond cleavage gave characteristic peak at 223 m/e. [3]
5. After C_5-C_6 bond cleavage, C_3-N_4 bond cleavage and subsequently N_4-C_5 bond cleavage gave two characteristic peaks at 208 m/e and 186 m/e respectively. [4] & [5]
6. After C_3-N_4 and N_4-C_5 bond cleavages, C_7-N_4 bond cleavage gave characteristic peak at 172 m/e. [6]
7. After C_7-N_4 bond cleavage, C_7-C_8 and C_8-C_9 bond cleavages gave two characteristic peaks at 158 m/e and 146 m/e (BASE PEAK). [7] & [8]
8. After C_7-C_8 and C_8-C_9 bond cleavages, C_9-N_{10} and C_{11}-C_{19} bond cleavages gave two characteristic peaks at 130 m/e and 118 m/e. [9] & [10]

9. N_4-C_7 bond cleavage in the title compound gave characteristic peak at 172 m/e, which could be the alternative possibility for this fragment. [11]
10. C_7-C_8 bond cleavage in the title compound gave two characteristic peaks at 158 m/e and 100 m/e, which could be the alternative possibility. [12]
11. C_8-C_9 bond cleavage in the title compound gave two characteristic peaks at 146 m/e (BASE PEAK) and 118 m/e, which could be the alternative possibility. [13]
12. C_9-N_{10} bond cleavage in the title compound gave characteristic peak at 130 m/e, which could be the alternative possibility. [14]

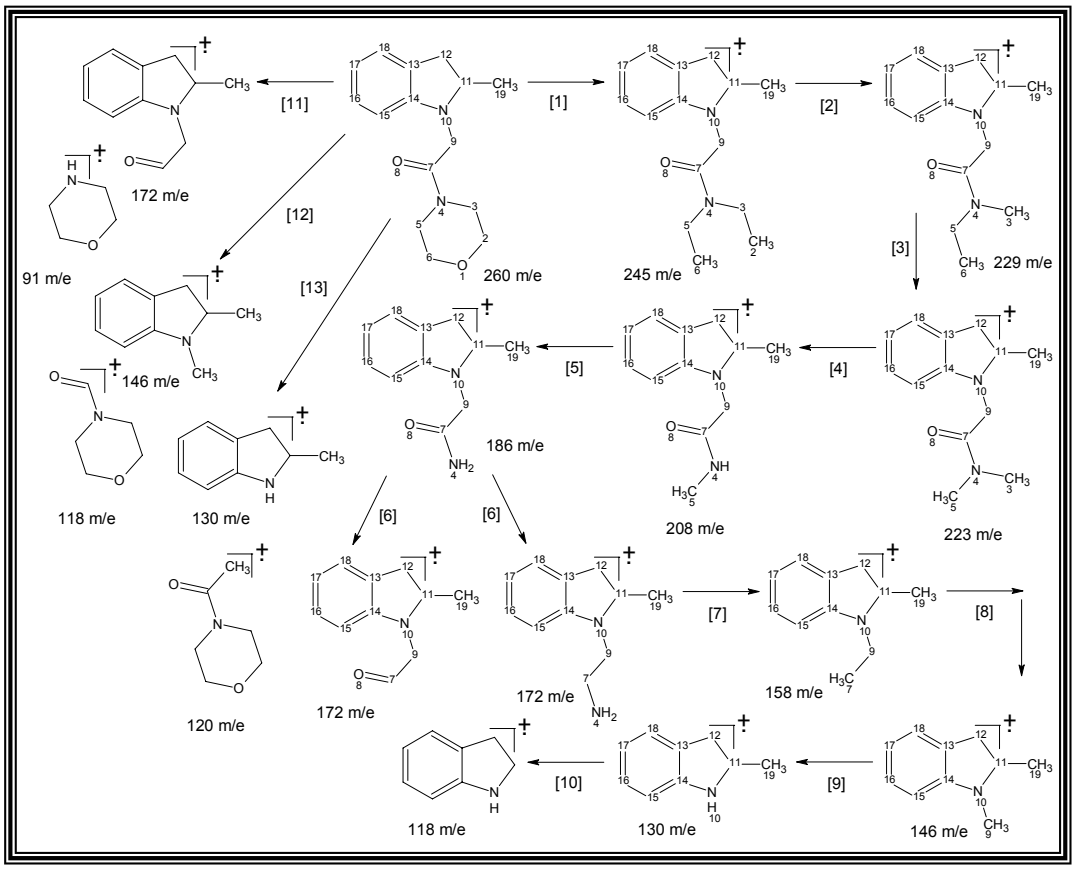
2.6.1.1 FRAGMENTATION PATTERN FOR DNJ-302



2-methyl-1-(2-morpholin-4-yl-2-oxoethyl) indoline (DNJ-402)

1. The target compound showed characteristic molecular ion peak.
2. O_1-C_2 and O_1-C_6 bond cleavages gave characteristic peak at 245 m/e. [1]
3. After O_1-C_2 and O_1-C_6 bond cleavages, C_2-C_3 bond cleavage gave characteristic peak at 229 m/e. [2]
4. After C_2-C_3 bond cleavage, C_5-C_6 bond cleavage gave characteristic peak at 223 m/e. [3]
5. After C_5-C_6 bond cleavage, C_3-N_4 bond cleavage and subsequently N_4-C_5 bond cleavage gave two characteristic peaks at 208 m/e and 186 m/e respectively. [4] & [5]
6. After C_3-N_4 and N_4-C_5 bond cleavages, C_7-N_4 bond cleavage or C_7-O_8 bond cleavage gave characteristic peak at 172 m/e which subsequently cleaved and gave peak at 158 m/e. [6] & [7]
7. C_7-C_9 bond cleavage gave characteristic peak at 146 m/e (BASE PEAK). [8]
8. After C_7-C_9 bond cleavage, C_9-N_{10} and C_{11}-C_{19} bond cleavages gave two characteristic peaks at 130 m/e and 118 m/e. [9] & [10]
9. N_4-C_7 bond cleavage in the title compound gave characteristic peak at 172 m/e, which could be the alternative possibility for this fragment. [11]
10. C_7-C_9 bond cleavage in the title compound gave two characteristic peaks at 146 m/e (BASE PEAK) and 118 m/e, which could be the alternative possibility. [12]
11. C_9-N_{10} bond cleavage in the title compound gave characteristic peak at 130 m/e, which could be the alternative possibility. [13]

2.6.1.2 FRAGMENTATION PATTERN FOR DNJ-402

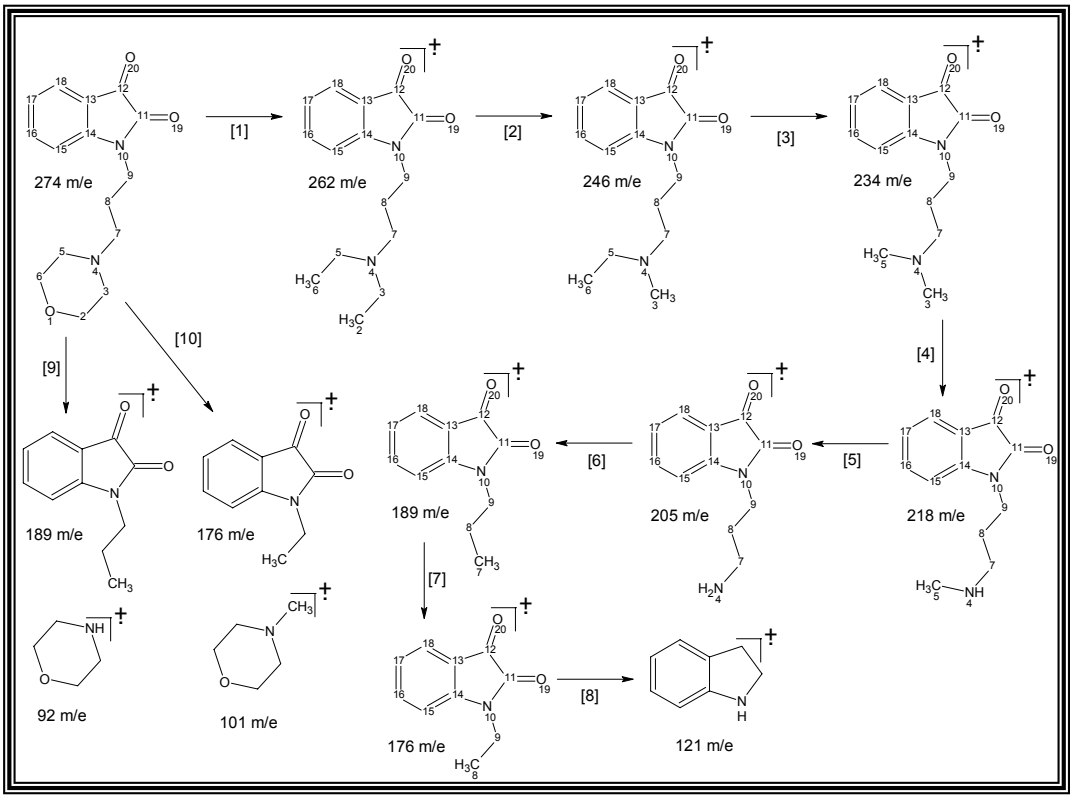


1-(3-morpholin-4-ylpropyl)-1*H*-indole-2, 3-dione (DNJ-502)

1. The target compound showed characteristic molecular ion peak.
2. O_1-C_2 and O_1-C_6 bond cleavages gave characteristic peak at 262 m/e. [1]
3. After O_1-C_2 and O_1-C_6 bond cleavages, C_2-C_3 bond cleavage gave characteristic peak at 246 m/e. [2]
4. After C_2-C_3 bond cleavage, C_5-C_6 bond cleavage gave characteristic peak at 234 m/e. [3]
5. After C_5-C_6 bond cleavage, C_3-N_4 bond cleavage and subsequently N_4-C_5 bond cleavage gave two characteristic peaks at 218 m/e and 205 m/e respectively. [4] & [5]

6. After C_3-N_4 and N_4-C_5 bond cleavages, C_7-N_4 bond cleavage gave characteristic peak at 189 m/e. [6]
7. After C_7-N_4 bond cleavage, C_7-C_8 bond cleavage gave characteristic peak at 176 m/e. [7]
8. After C_7-C_8 bond cleavage, C_9-N_{10} and subsequently C_{11}-C_{19} and C_{12}-C_{20} bond cleavages gave characteristic peak at 121 m/e. [8]
9. N_4-C_7 bond cleavage in the title compound gave characteristic peak at 189 m/e, which could be the alternative possibility for this fragment. [9]
10. C_7-C_8 bond cleavage in the title compound gave two characteristic peaks at 176 m/e and 101 m/e, which could be the alternative possibility. [10]

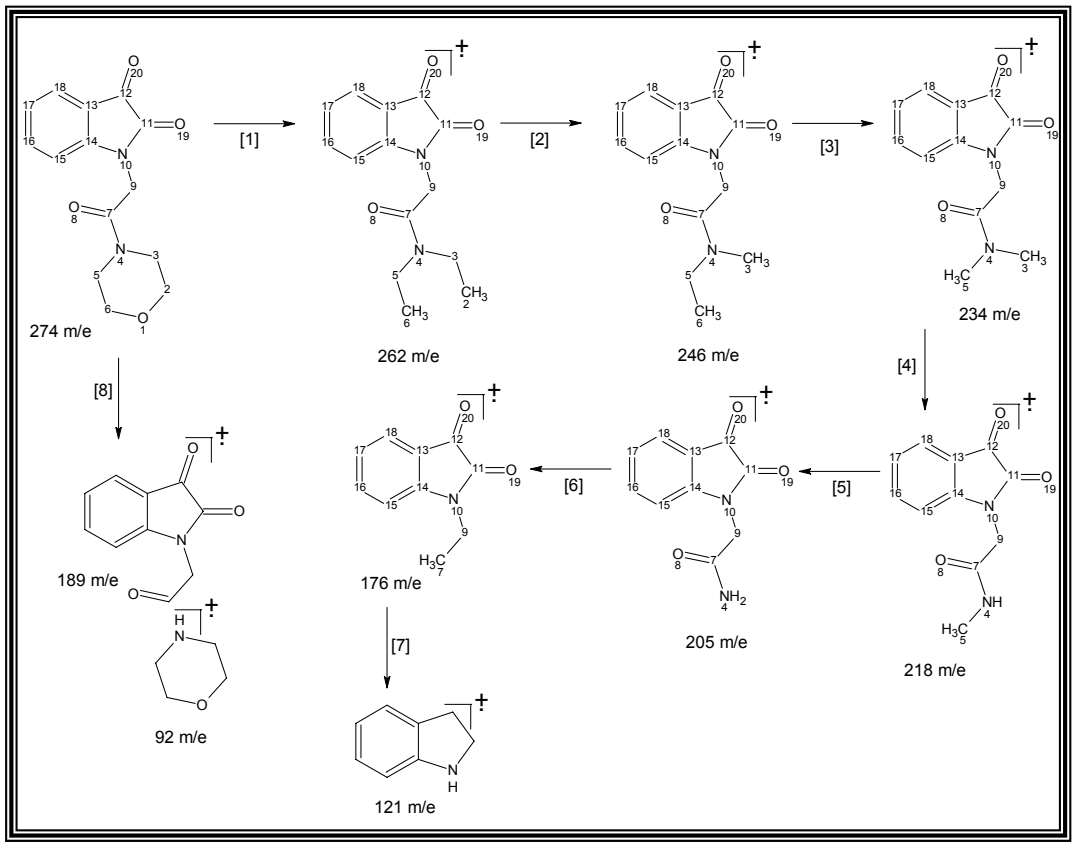
2.6.1.3 FRAGMENTATION PATTERN FOR DNJ-502



1-(2-morpholin-4-yl-2-oxoethyl)-1*H*-indole-2, 3-dione (DNJ-602)

1. The target compound showed characteristic molecular ion peak.
2. O_1-C_2 and O_1-C_6 bond cleavages gave characteristic peak at 262 m/e. [1]
3. After O_1-C_2 and O_1-C_6 bond cleavages, C_2-C_3 bond cleavage gave characteristic peak at 246 m/e. [2]
4. After C_2-C_3 bond cleavage, C_5-C_6 bond cleavage gave characteristic peak at 234 m/e. [3]
5. After C_5-C_6 bond cleavage, C_3-N_4 bond cleavage and subsequently N_4-C_5 bond cleavage gave two characteristic peaks at 218 m/e and 205 m/e respectively. [4] & [5]
6. After C_3-N_4 and N_4-C_5 bond cleavages, N_4-C_7 or C_7-O_8 bond cleavage gave characteristic peak at 176 m/e. [6]
7. Afterwards C_9-N_{10} and subsequently C_{11}-C_{19} and C_{12}-C_{20} bond cleavages gave characteristic peak at 121 m/e. [7]
8. N_4-C_7 bond cleavage in the title compound gave characteristic peak at 189 m/e, which could be the alternative possibility for this fragment. [8]

2.6.1.4 FRAGMENTATION PATTERN FOR DNJ-602



2.6.2 IR SPECTRAL STUDY

IR spectra of the synthesized compounds were recorded on **Shimadzu FT IR 8400** spectrophotometer using Diffused Reflectance Attachment (DRA) System using Potassium Bromide.

In case of DNJ-301 to DNJ-305, aromatic C-H stretching and bending frequencies were found near 3050 cm^{-1} and 1460 cm^{-1} respectively. C-H stretching frequencies for methyl and methylene group were obtained near 2950 cm^{-1} and 2850 cm^{-1} respectively. Aliphatic C-N vibrations were found in the region of 1020-1220 cm^{-1}. Characteristic frequency for ether linkage was also found near 1050 cm^{-1} in DNJ-302. Along with all the frequencies obtained

in above compounds, additionally carbonyl frequency was found in DNJ-401 to DNJ-405, DNJ-501 to DNJ-505 and DNJ-601 to DNJ-605 compounds.

2.6.3 ^{1}H NMR SPECTRAL STUDY

^{1}H & ^{13}C NMR spectra of the synthesized compounds were recorded on **Bruker Avance II 400** spectrometer. Sample solutions were made in $CDCl_3$ solvent using tetramethylsilane (TMS) as the internal standard unless otherwise mentioned. Numbers of protons and numbers of carbons identified from H NMR & C NMR spectrum and their chemical shift (δ ppm) were in the agreement of the structure of the molecule. *J* values were calculated to identify o, m and p coupling. In some cases, aromatic protons were obtained as multiplet. ^{1}H & ^{13}C NMR spectral interpretation can be discussed as under.

^{1}H NMR spectral interpretation of 2-methyl-1-(3-morpholin-4-ylpropyl) indoline (DNJ-302)

1. Three most shielded protons of methyl group (C_{19}) gave multiplet at 1.28 δ ppm. Usually these protons should show their multiplicity as doublet due to the presence of single proton at C_{11}, but two nitrogen atoms are present in the molecule and thus due to their effect these methyl protons coupled with one proton of methine group (C_{11}) and another two protons of methylene group (C_{12}) and gave multiplet.
2. Two methylene protons of propyl chain (C_8) gave multiplet at 2.20 δ ppm due to the presence of two methylene carbons (C_7 & C_9).
3. Four protons of morpholinyl methylene carbons (C_3 & C_5) and two protons of methylene carbon of propyl chain (C_7) gave multiplet at 3.31 δ ppm.
4. Two methylene protons of propyl chain (C_9) gave triplet at 3.54 δ ppm.
5. One proton of methine group (C_{11}) gave quartet at 4.32 δ ppm which actually should show multiplet due to the presence of three protons of methyl group (C_{19}) and two protons of methylene group (C_{12}).

6. Four protons of morpholinyl methylene carbons (C_2 & C_6) gave triplet at 5.00 δ ppm.
7. Two aromatic protons of C_{15} and C_{17} methine groups gave multiplet at 6.63 δ ppm while another two aromatic protons of C_{16} and C_{18} methine groups gave multiplet at 7.00 δ ppm.

^{1}H NMR spectral interpretation of 2-methyl-1-(2-oxo-2-piperidin-1-ylethyl) indoline (DNJ-402)

1. Three most shielded protons of methyl group (C_{13}) gave multiplet at 1.28 δ ppm. Usually these protons should show their multiplicity as doublet due to the presence of single proton at C_2, but two nitrogen atoms are present in the molecule and thus due to their effect these methyl protons coupled with one proton of methine group (C_2) and another two protons of methylene group (C_3) and gave multiplet.
2. Four protons of two morpholinyl methylene groups attached with the nitrogen atom gave triplet at 2.45 δ ppm, while another four protons of rest of the two morpholinyl methylene groups attached with the oxygen atom gave triplet at 3.73 δ ppm.
3. Two protons of C_3 carbon atom splitted into two which showed singlet for each proton at 2.62 δ ppm and at 2.83 δ ppm respectively.
4. One proton of methine group (C_2) gave quartet at 3.14 δ ppm which actually should show multiplet due to the presence of three protons of methyl group (C_{13}) and two protons of methylene group (C_3).
5. Two protons of methylene group (C_{10}) became deshielded due to the two nitrogen atoms and gave singlet at 4.44 δ ppm.
6. Two aromatic protons of C_6 and C_8 methine groups gave multiplet at 6.63 δ ppm while another two aromatic protons of C_7 and C_9 methine groups gave multiplet at 7.00 δ ppm.

^{1}H NMR spectral interpretation of 1-(3-morpholin-4-ylpropyl)-1*H*-indole-2, 3-dione (DNJ-502)

1. Two methylene protons of C_8 gave multiplet at 1.28 δ ppm.

2. Six protons of three methylene carbons (C_3, C_5 and C_7) gave multiplet at 2.83 δ ppm.
3. Two protons of methylene carbon (C_9) of propyl chain gave triplet at 3.73 δ ppm.
4. Four protons of two morpholinyl methylene carbons (C_2 & C_6) gave triplet at 4.52 δ ppm.
5. Two aromatic protons of C_{15} and C_{17} methine groups gave multiplet at 6.63 δ ppm while another two aromatic protons of C_{16} and C_{18} methine groups gave multiplet at 7.00 δ ppm.

^{1}H NMR spectral interpretation of 1-(2-oxo-2-piperidin-1-ylethyl)-1*H*-indole-2, 3-dione (DNJ-602)

1. Four protons of two morpholinyl carbon atoms (C_3 & C_5) gave triplet at 2.61 δ ppm.
2. Four protons of two morpholinyl carbon atoms (C_3 & C_6) gave triplet at 3.95 δ ppm.
3. Two protons of methylene carbon atom (C_9) gave singlet at 4.47 δ ppm.
4. Two aromatic protons of C_{15} and C_{17} methine groups gave multiplet at 6.63 δ ppm while another two aromatic protons of C_{16} and C_{18} methine groups gave multiplet at 7.00 δ ppm.

2.6.4 ELEMENTAL ANALYSIS

Elemental analysis of the synthesized compounds was carried out on **Vario EL Carlo Erba 1108** which showed calculated and found percentage values of Carbon, Hydrogen and Nitrogen in support of the structure of synthesized compounds. The spectral and elemental analysis data are given for individual compounds.

2.7 ANALYTICAL DATA

2-METHYL-1-(3-PIPERIDIN-1-YLPROPYL) INDOLINE (DNJ-301): IR (KBr, cm^{-1}): 3060 (Ar-H), 2960 (–CH_3), 2852 (–CH_2), 1607, 1505, 1477, 1452 (Ar-H), 1120 (C-N, Aliphatic, 3°); MS m/z = 258 (M^+); Anal. Calcd. for $C_{17}H_{26}N_2$: C, 79.02; H, 10.14; N, 10.84. Found: C, 79.06; H, 10.12; N, 10.82.

2-METHYL-1-(3-MORPHOLIN-4-YLPROPYL) INDOLINE (DNJ-302): IR (KBr, cm^{-1}): 3106 (Ar-H), 2949 (–CH_3), 2855 (–CH_2), 1465 (Ar-H), 1112 (C-N, Aliphatic, 3°), 1050 (C-O-C); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.28 (3H, m), 2.20 (2H, m), 3.31 (8H, m), 3.54 (2H, t), 4.32 (1H, qt), 5.00 (4H, t), 6.63 (2H, m), 7.00 (2H, m); MS m/z = 260 (M^+); Anal. Calcd. for $C_{16}H_{24}N_2O$: C, 73.81; H, 9.29; N, 10.76. Found: C, 73.84; H, 9.32; N, 10.80.

2-METHYL-1-[3-(4-METHYL PIPERAZIN-1-YL) PROPYL] INDOLINE (DNJ-303): IR (KBr, cm^{-1}): 3055 (Ar-H), 2958 (–CH_3), 2847 (–CH_2), 1620, 1500, 1460 (Ar-H), 1135 (C-N, Aliphatic, 3°); MS m/z = 273 (M^+); Anal. Calcd. for $C_{17}H_{27}N_3$: C, 74.68; H, 9.95; N, 15.37. Found: C, 74.65; H, 9.97; N, 15.38.

1-[3-(4-ETHYL PIPERAZIN-1-YL) PROPYL]-2-METHYL INDOLINE (DNJ-304): IR (KBr, cm^{-1}): 3068 (Ar-H), 2945 (–CH_3), 2849 (–CH_2), 1630, 1524, 1462 (Ar-H), 1129 (C-N, Aliphatic, 3°); MS m/z = 287 (M^+); Anal. Calcd. for $C_{18}H_{29}N_3$: C, 75.21; H, 10.17; N, 14.62. Found: C, 75.17; H, 10.19; N, 14.64.

1-[3-(4-BENZYL PIPERAZIN-1-YL) PROPYL]-2-METHYL INDOLINE (DNJ-305): IR (KBr, cm^{-1}): 3057 (Ar-H), 2950 (–CH_3), 2866 (–CH_2), 1624, 1500, 1455, 1423 (Ar-H), 1130 (C-N, Aliphatic, 3°); MS m/z = 349 (M^+); Anal. Calcd. for $C_{23}H_{31}N_3$: C, 79.04; H, 8.94; N, 12.02. Found: C, 79.03; H, 8.96; N, 12.01.

2-METHYL-1-(2-OXO-2-PIPERIDIN-1-YL ETHYL) INDOLINE (DNJ-401): IR (KBr, cm^{-1}): 3079 (Ar-H), 2951 (–CH_3), 2872 (–CH_2), 1727 (>CO), 1630, 1542, 1477, 1439 (Ar-H), 1140 (C-N, Aliphatic, 3°); MS m/z = 258 (M^+); Anal. Calcd.

for $C_{16}H_{22}N_2O$: C, 74.38; H, 8.58; N, 10.84. Found: C, 74.34; H, 8.54; N, 10.80.

2-METHYL-1-(2-MORPHOLIN-4-YL-2-OXO ETHYL) INDOLINE (DNJ-402): IR (KBr, cm^{-1}): 3110 (Ar-H), 2949 ($–CH_3$), 2855 ($–CH_2$), 1735 (>CO), 1613, 1469, 1439 (Ar-H), 1113 (C-N, Aliphatic, 3°), 1050 (C-O-C); ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.28 (3H, m), 2.45 (4H, t), 2.62 (1H, s), 2.83 (1H, s), 3.14 (1H, s), 3.73 (4H, t), 4.44 (2H, s), 6.63 (2H, m), 7.00 (2H, m); MS m/z = 260 (M^+); Anal. Calcd. for $C_{15}H_{20}N_2O_2$: C, 69.20; H, 7.74; N, 10.76. Found: C, 69.24; H, 7.70; N, 10.80.

2-METHYL-1-[2-(4-METHYL PIPERAZIN-1-YL)-2-OXO ETHYL] INDOLINE (DNJ-403): IR (KBr, cm^{-1}): 3082 (Ar-H), 2964 ($–CH_3$), 2859 ($–CH_2$), 1730 (>CO), 1627, 1534, 1480, 1423 (Ar-H), 1120 (C-N, Aliphatic, 3°); MS m/z = 273 (M^+); Anal. Calcd. for $C_{16}H_{23}N_3O$: C, 70.30; H, 8.48; N, 15.37. Found: C, 70.26; H, 8.46; N, 15.40.

1-[2-(4-ETHYL PIPERAZIN-1-YL)-2-OXO ETHYL]-2-METHYL INDOLINE (DNJ-404): IR (KBr, cm^{-1}): 3105 (Ar-H), 2969 ($–CH_3$), 2848 ($–CH_2$), 1722 (>CO), 1662, 1500, 1447 (Ar-H), 1110 (C-N, Aliphatic, 3°); MS m/z = 287 (M^+); Anal. Calcd. for $C_{17}H_{25}N_3O$: C, 71.04; H, 8.77; N, 14.62. Found: C, 71.08; H, 8.80; N, 14.65.

1-[2-(4-BENZYL PIPERAZIN-1-YL)-2-OXO ETHYL]-2-METHYL INDOLINE (DNJ-405): IR (KBr, cm^{-1}): 3068 (Ar-H), 2963 ($–CH_3$), 2865 ($–CH_2$), 1730 (>CO), 1620, 1470, 1445 (Ar-H), 1130 (C-N, Aliphatic, 3°); MS m/z = 349 (M^+); Anal. Calcd. for $C_{22}H_{27}N_3O$: C, 75.61; H, 7.79; N, 12.02. Found: C, 75.57; H, 7.82; N, 12.05.

1-(3-PIPERIDIN-1-YL PROPYL)-1*H*-INDOLE-2, 3-DIONE (DNJ-501): IR (KBr, cm^{-1}): 3100 (Ar-H), 2963 ($–CH_3$), 2846 ($–CH_2$), 1724 (>CO, C_{12}), 1680 (>CO, C_{11}), 1610, 1555, 1448 (Ar-H), 1120 (C-N, Aliphatic, 3°); MS m/z = 272 (M^+); Anal. Calcd. for $C_{16}H_{20}N_2O_2$: C, 70.56; H, 7.40; N, 10.29. Found: C, 70.60; H, 7.42; N, 10.32.

1-(3-MORPHOLIN-4-YL PROPYL)-1*H*-INDOLE-2, 3-DIONE (DNJ-502): IR (KBr, cm^{-1}): 3137 (Ar-H), 2953 ($–CH_3$), 2863 ($–CH_2$), 1730 (>CO, C_{12}), 1682 (>CO, C_{11}), 1609, 1562, 1461 (Ar-H), 1118 (C-N, Aliphatic, 3°), 1050 (C-O-C); ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.28 (2H, m), 2.83 (6H, m), 3.73 (2H, t), 4.52 (4H, t), 6.63 (2H, m), 7.00 (2H, m); MS m/z = 274 (M^+); Anal. Calcd. for $C_{15}H_{18}N_2O_3$: C, 65.68; H, 6.61; N, 10.21. Found: C, 65.70; H, 6.64; N, 10.24.

1-[3-(4-METHYL PIPERAZIN-1-YL) PROPYL]-1*H*-INDOLE-2, 3-DIONE (DNJ-503): IR (KBr, cm^{-1}): 3090 (Ar-H), 2969 ($–CH_3$), 2855 ($–CH_2$), 1721 (>CO, C_{12}), 1683 (>CO, C_{11}), 1600, 1550, 1433 (Ar-H), 1127 (C-N, Aliphatic, 3°); MS m/z = 287 (M^+); Anal. Calcd. for $C_{16}H_{21}N_3O_2$: C, 66.88; H, 7.37; N, 14.62. Found: C, 66.90; H, 7.40; N, 14.65.

1-[3-(4-ETHYL PIPERAZIN-1-YL) PROPYL]-1*H*-INDOLE-2, 3-DIONE (DNJ-504): IR (KBr, cm^{-1}): 3081 (Ar-H), 2950 ($–CH_3$), 2861 ($–CH_2$), 1729 (>CO, C_{12}), 1685 (>CO, C_{11}), 1627, 1544, 1433 (Ar-H), 1115 (C-N, Aliphatic, 3°); MS m/z = 301 (M^+); Anal. Calcd. for $C_{17}H_{23}N_3O_2$: C, 67.75; H, 7.69; N, 13.94. Found: C, 67.71; H, 7.66; N, 13.91.

1-[3-(4-BENZYL PIPERAZIN-1-YL) PROPYL]-1*H*-INDOLE-2, 3-DIONE (DNJ-505): IR (KBr, cm^{-1}): 3075 (Ar-H), 2960 ($–CH_3$), 2840 ($–CH_2$), 1730 (>CO, C_{12}), 1688 (>CO, C_{11}), 1624, 1575, 1525, 1440 (Ar-H), 1122 (C-N, Aliphatic, 3°); MS m/z = 363 (M^+); Anal. Calcd. for $C_{22}H_{25}N_3O_2$: C, 72.70; H, 6.93; N, 11.56. Found: C, 72.68; H, 6.95; N, 11.60.

1-(PIPERIDIN-1-YL ACETYL)-1*H*-INDOLE-2, 3-DIONE (DNJ-601): IR (KBr, cm^{-1}): 3080 (Ar-H), 2963 ($–CH_3$), 2854 ($–CH_2$), 1723 (>CO, C_{12}), 1687 (>CO, C_{11}), 1663 (>CO, C_7), 1615, 1519, 1450 (Ar-H), 1125 (C-N, Aliphatic, 3°); MS m/z = 272 (M^+); Anal. Calcd. for $C_{15}H_{16}N_2O_3$: C, 66.16; H, 5.92; N, 10.29. Found: C, 66.20; H, 5.89; N, 10.31.

1-(MORPHOLIN-4-YL ACETYL)-1*H*-INDOLE-2, 3-DIONE (DNJ-602): IR (KBr, cm^{-1}): 3069 (Ar-H), 2953 ($–CH_3$), 2864 ($–CH_2$), 1713 (>CO, C_{12}), 1682 (>CO, C_{11}), 1659 (>CO, C_7), 1609, 1519, 1454 (Ar-H), 1117 (C-N, Aliphatic,

3°), 1048 (C-O-C); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 2.61 (4H, t), 3.95 (4H, t), 4.47 (2H, s), 6.63 (2H, m), 7.00 (2H, m); MS m/z = 274 (M^+); Anal. Calcd. for $C_{14}H_{14}N_2O_4$: C, 61.31; H, 5.14; N, 10.21. Found: C, 61.35; H, 5.10; N, 10.24.

1-[(4-METHYL PIPERAZIN-1-YL) ACETYL]-1*H*-INDOLE-2, 3-DIONE (DNJ-603): IR (KBr, cm^{-1}): 3070 (Ar-H), 2945 (–CH_3), 2860 (–CH_2), 1710 (>CO, C_{12}), 1678 (>CO, C_{11}), 1655 (>CO, C_7), 1600, 1588, 1524, 1450 (Ar-H), 1111 (C-N, Aliphatic, 3°); MS m/z = 287 (M^+); Anal. Calcd. for $C_{15}H_{17}N_3O_3$: C, 62.71; H, 5.96; N, 14.63. Found: C, 62.75; H, 5.92; N, 14.60.

1-[(4-ETHYL PIPERAZIN-1-YL) ACETYL]-1*H*-INDOLE-2, 3-DIONE (DNJ-604): IR (KBr, cm^{-1}): 3064 (Ar-H), 2962 (–CH_3), 2860 (–CH_2), 1720 (>CO, C_{12}), 1685 (>CO, C_{11}), 1650 (>CO, C_7), 1600, 1570, 1500, 1455 (Ar-H), 1123 (C-N, Aliphatic, 3°); MS m/z = 301 (M^+); Anal. Calcd. for $C_{16}H_{19}N_3O_3$: C, 63.77; H, 6.36; N, 13.94. Found: C, 63.80; H, 6.33; N, 13.91.

1-[(4-BENZYL PIPERAZIN-1-YL) ACETYL]-1*H*-INDOLE-2, 3-DIONE (DNJ-605): IR (KBr, cm^{-1}): 3077 (Ar-H), 2950 (–CH_3), 2869 (–CH_2), 1715 (>CO, C_{12}), 1680 (>CO, C_{11}), 1662 (>CO, C_7), 1612, 1530, 1466, 1420 (Ar-H), 1122 (C-N, Aliphatic, 3°); MS m/z = 363 (M^+); Anal. Calcd. for $C_{21}H_{21}N_3O_3$: C, 69.41; H, 5.82; N, 11.56. Found: C, 69.45; H, 5.85; N, 11.60.

2.8 RESULTS AND DISCUSSION

Reactions under dry conditions (i.e., in the absence of a solvent, on a solid support with or without catalysts) were originally developed in the late 1980s. Synthesis without solvents under microwave irradiation offers several advantages. The absent of solvent reduces the risk of explosions when the reaction takes place in a closed vessel in an oven. Moreover, aprotic dipolar solvents with high boiling points are expensive and difficult to remove from the reaction mixtures. During microwave induction of reactions under dry conditions, the reactants adsorbed on the surface of alumina, silica gel, clay, and others absorb microwaves whereas the support does not, nor does it restrict the transmission of microwaves. Consequently, such supported reagents efficiently induce reactions under safe and simple conditions with domestic microwave ovens instead of specialized expensive commercial microwave systems.

The *N*-alkylation of heterocyclic compounds bearing an acidic hydrogen atom attached to nitrogen is generally accomplished by treatment of these compounds with an appropriate base followed by treatment of the resulting salts with an alkylating agent.

In this chapter, dry conditions have been used in the synthesis of all the organic compounds. Anhydrous potassium carbonate was used as a solid support and as a basic media too. Dimethylformamide was used in very little amount to make a slurry at room temperature which has not been considered as a solvent. Household microwave oven (LG MS-192 W) was used as a microwave source operating range of 800 W. All the reactions were carried out at 480 W for desired time.

There are so many reported methods in the literature for the *N*-alkylation conventionally as well as microwave induced. Conventional methods are having tedious work up and purification procedures and are time consuming while microwave assisted methods are straight forward, fast, time

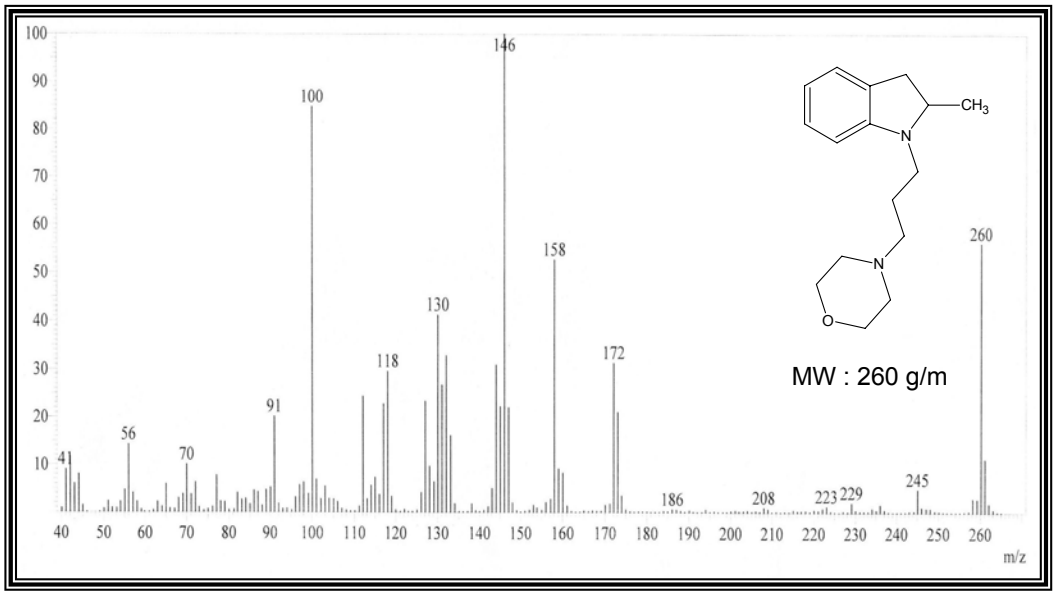
saving and eco-friendly. So in this chapter microwave induced dry method has been developed which produced high yielding end products.

2.9 CONCLUSION

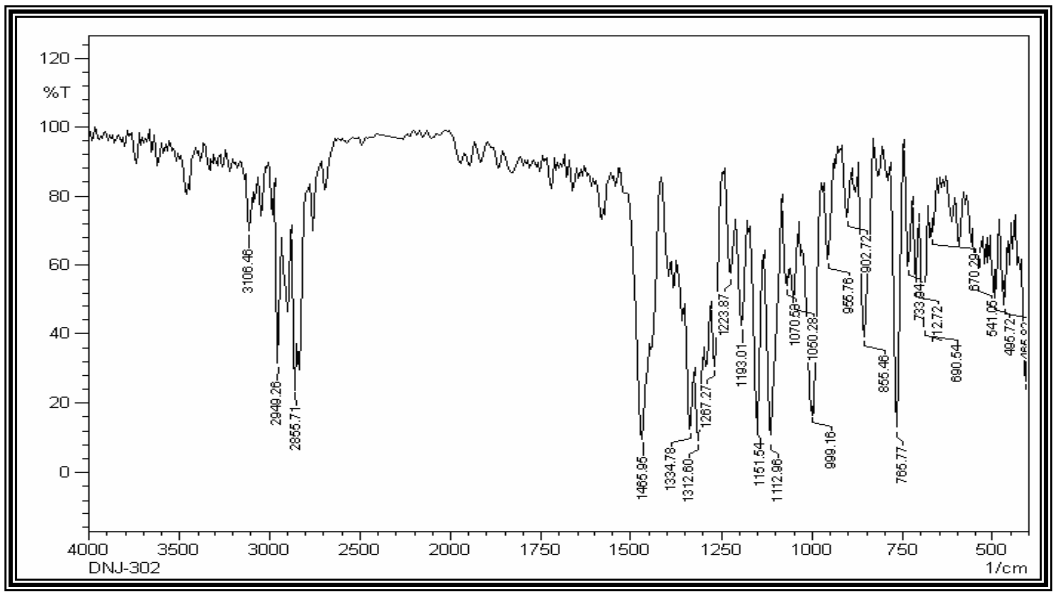
Looking to the importance of indole nucleus and diversified biological profile shown by different indole derivatives, study on different 2-methyl indoline and isatin derivatives has been continued in this chapter. More over importance and advantages of microwave assisted organic synthesis attracted us to contribute in green chemistry by developing eco-friendly methods for the preparation of organic molecules. *N*-alkylation is one of the most important reactions to be carried out on different substrates from chemistry point of view as well as biological activity point of view. In current chapter, not only new and novel molecules have been prepared but simple and fast microwave assisted *N*-alkylation method is also developed.

2.10 SPECTRAL REPRESENTATION OF SYNTHESIZED COMPOUNDS

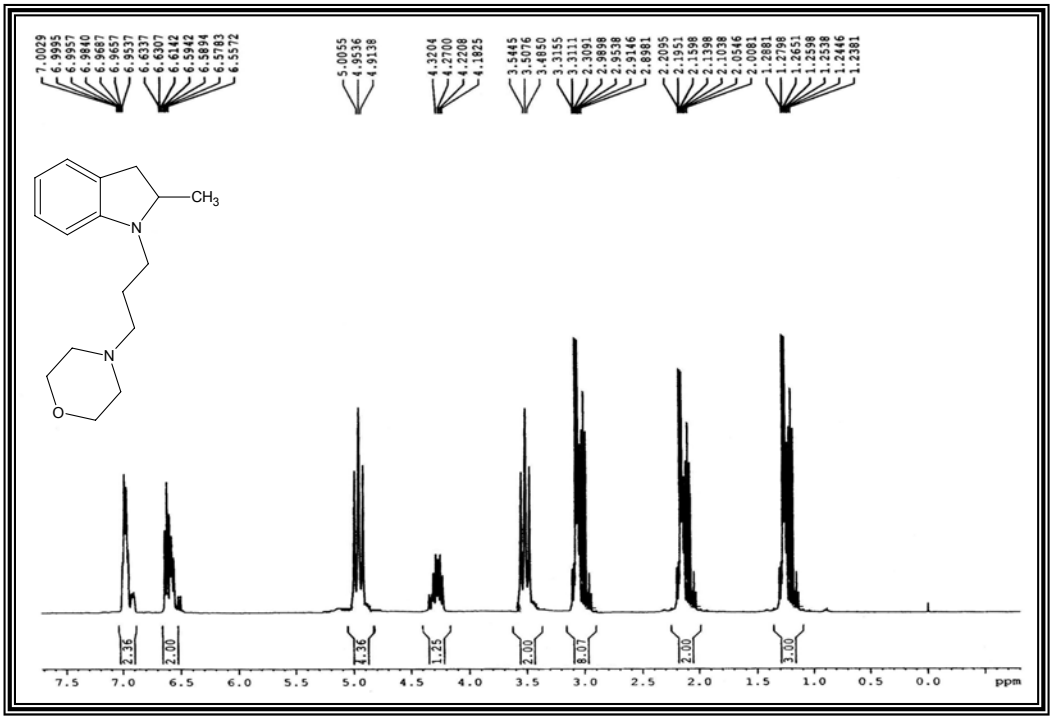
2.10.1 Mass Spectrum of DNJ-302



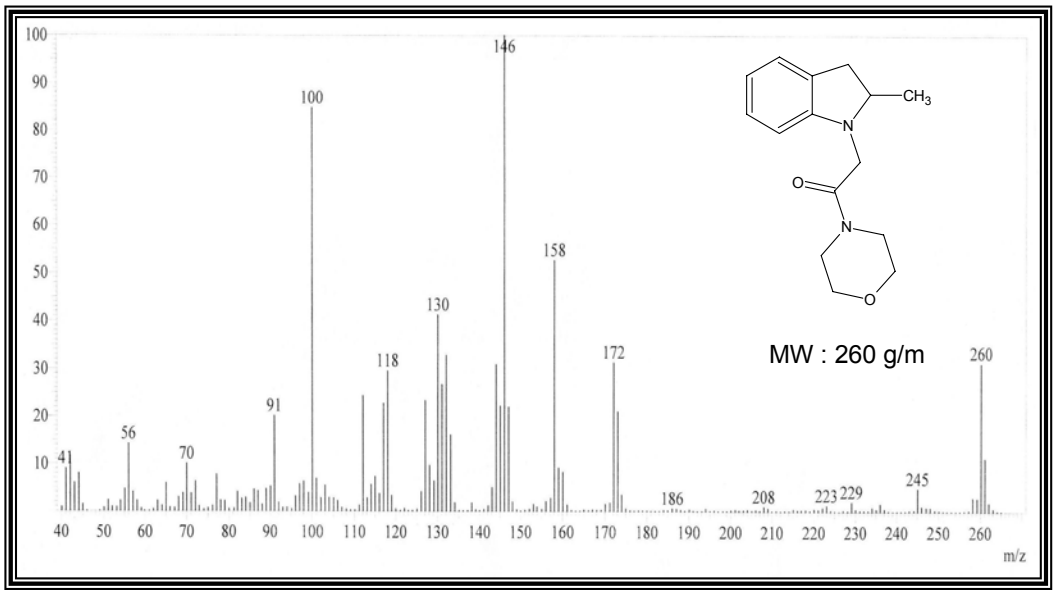
2.10.2 IR Spectrum of DNJ-302



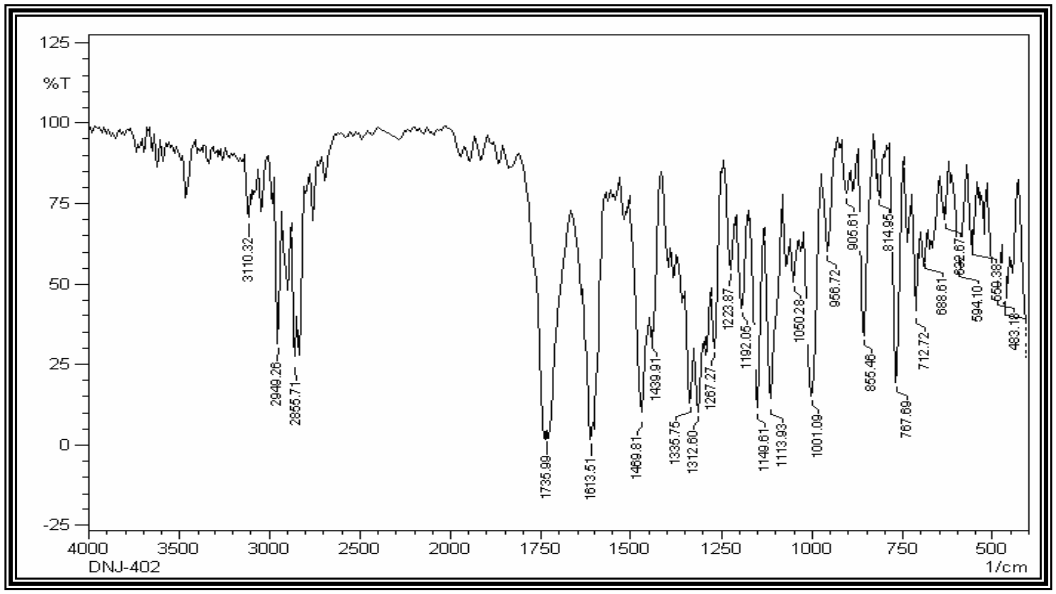
2.10.3 ^{1}H NMR Spectrum of DNJ-302



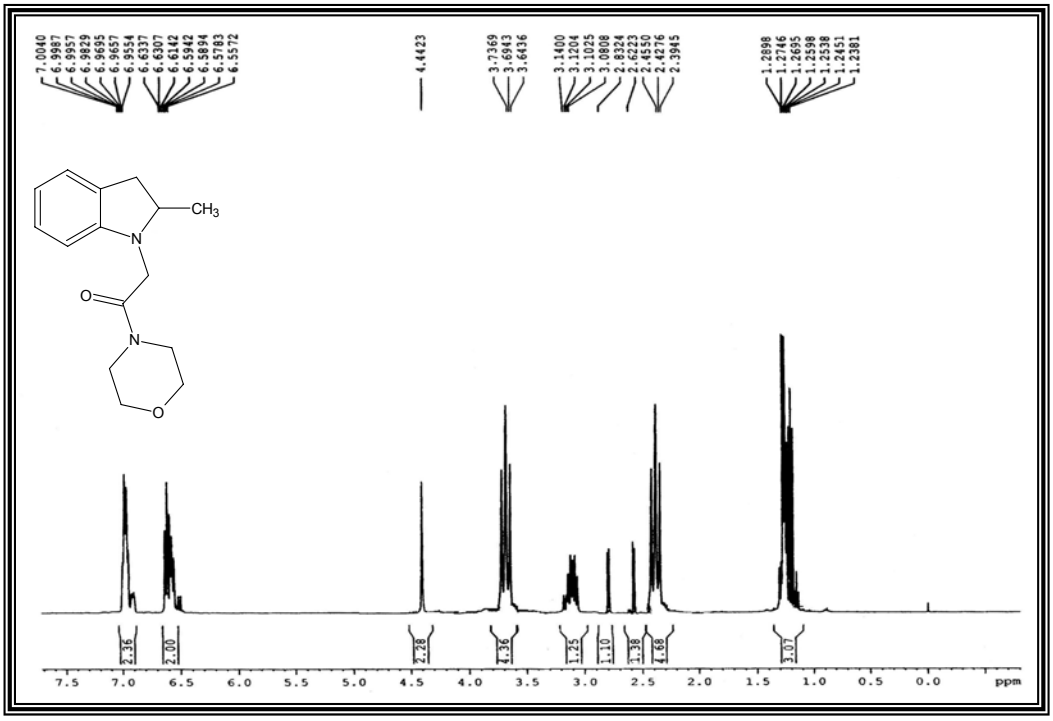
2.10.4 Mass Spectrum of DNJ-402



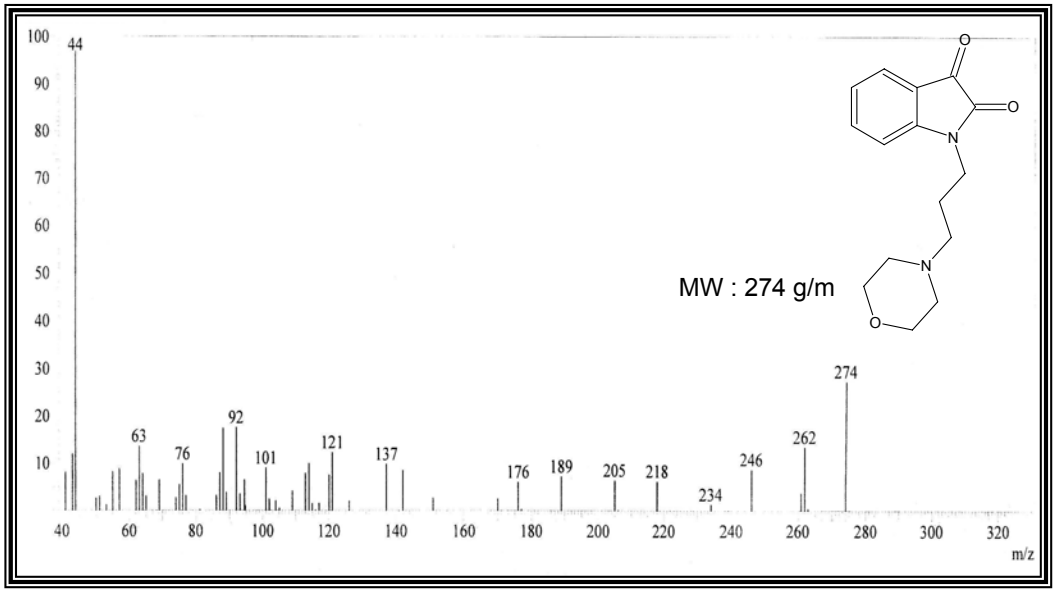
2.10.5 IR Spectrum of DNJ-402



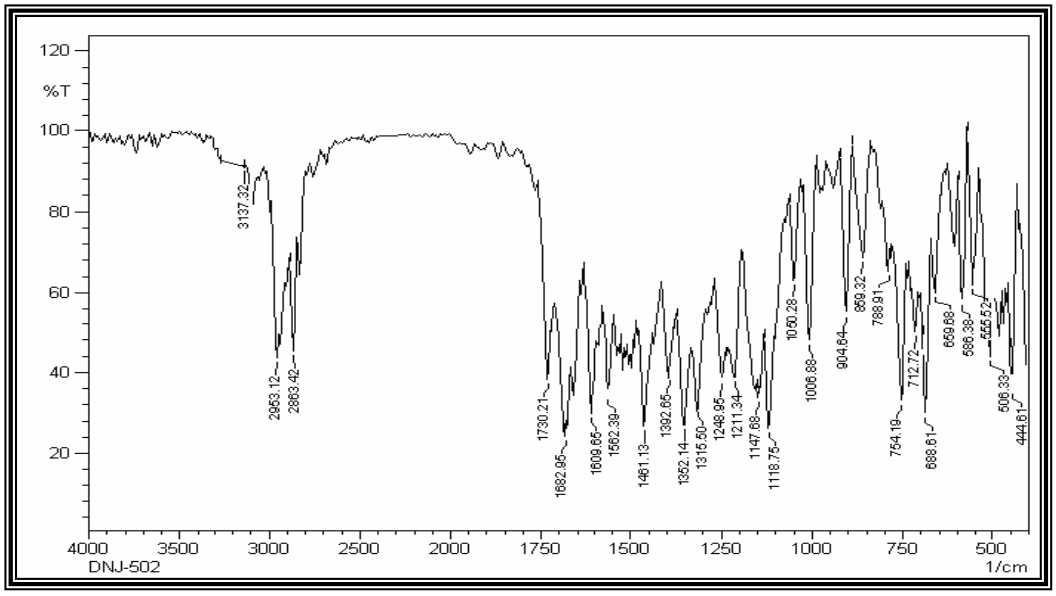
2.10.6 ^{1}H NMR Spectrum of DNJ-402



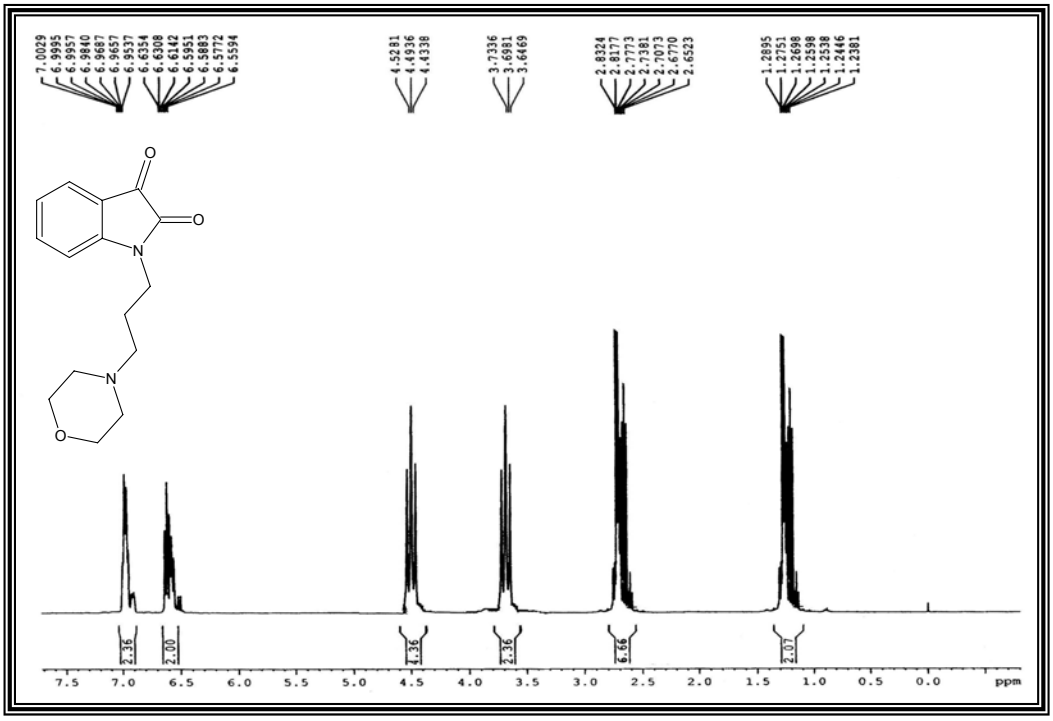
2.10.7 Mass Spectrum of DNJ-502



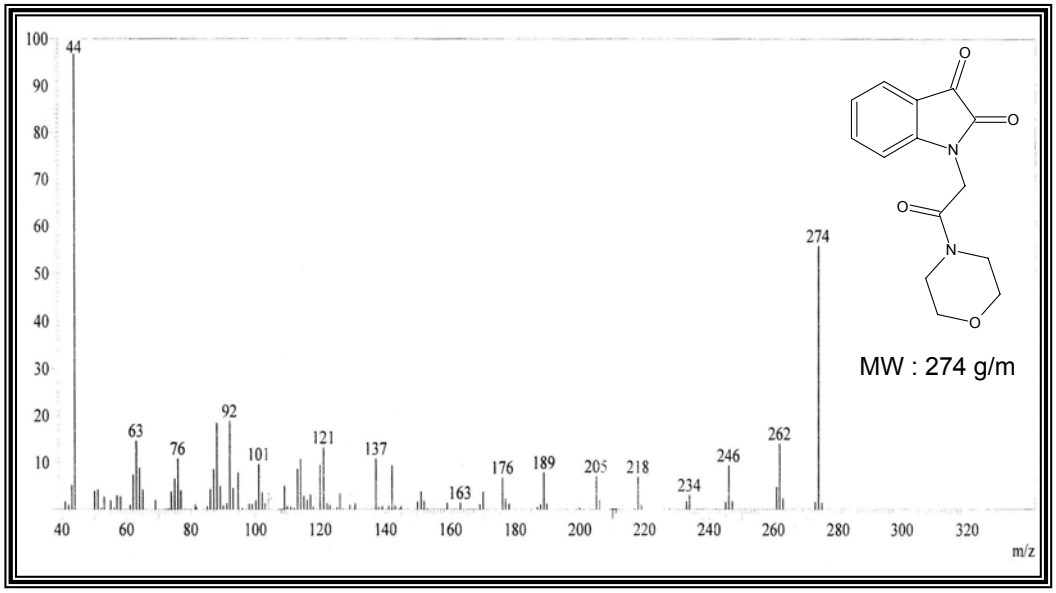
2.10.8 IR Spectrum of DNJ-502



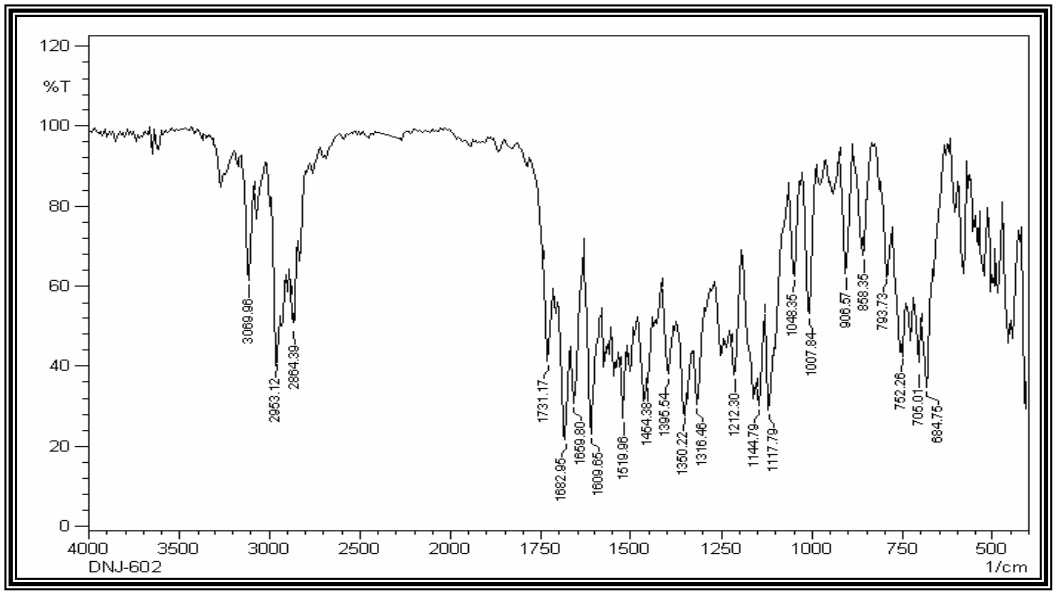
2.10.9 ^{1}H NMR Spectrum of DNJ-502



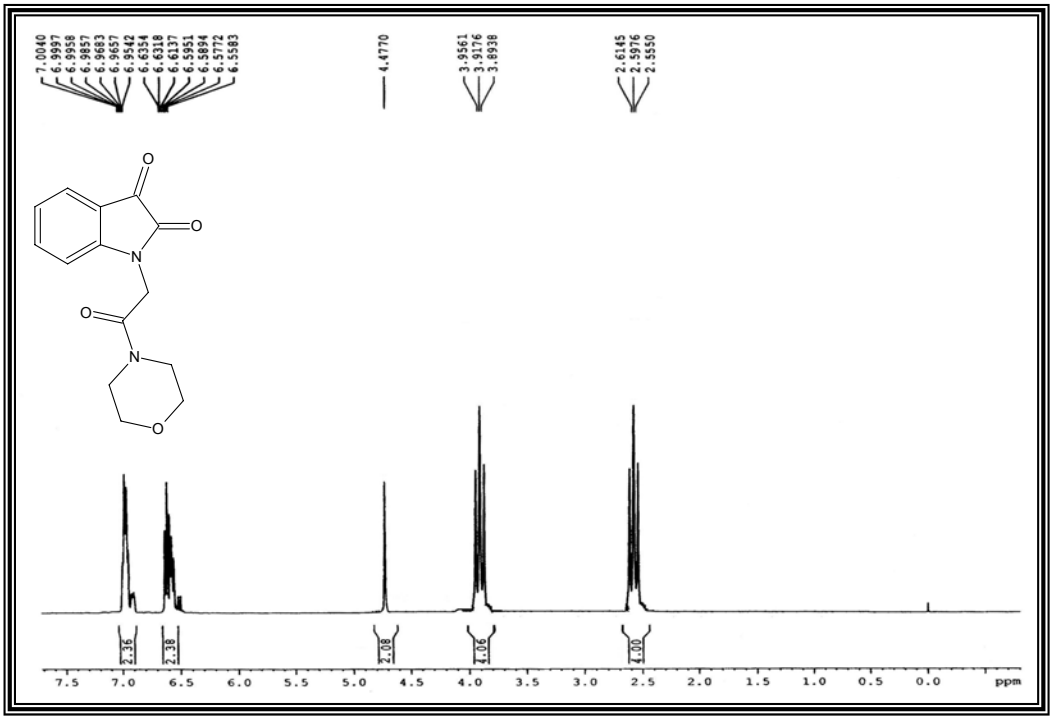
2.10.10 Mass Spectrum of DNJ-602



2.10.11 IR Spectrum of DNJ-602



2.10.12 ^{1}H NMR Spectrum of DNJ-602



PART – B

STUDIES ON ISATIN DERIVATIVES

B.1 INTRODUCTION TO ISATIN

Isatin (1*H*-indole-2, 3-dione; $C_8H_5NO_2$) is an aromatic heterocyclic organic compound. It has a bicyclic structure, consisting of a six-membered benzene ring fused to a five-membered nitrogen-containing ring. The compound's structure is based on the indoline structure but where two carbonyl groups are situated at 2- and 3-position of the five membered ring.

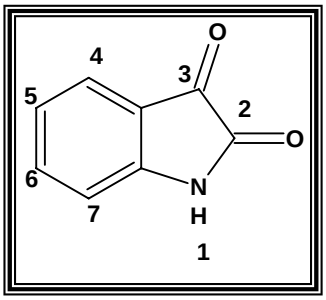


Fig. B.1.1

In the course of studies of the action of various oxidizing agents on indigo, Erdmann [1-3] and Laurent [4-7] in 1841 independently discovered an oxidation product, which had the formula $C_8H_6NO_2$ and to which the name given was isatin.

In nature, isatin is found in plants of the genus *Isatis*, [8] in *Calanthe discolor* LINDL. [9] and in *Couroupita guianensis* Aubl. [10] and has also been found as a component of the secretion from the parotid gland of *Bufo* frogs, [11] and in humans as it is a metabolic derivative of adrenaline. [12-14] Substituted isatins are also found in plants, for example the melosatin alkaloids (methoxy phenylpentyl isatins) obtained from the Caribbean tumorigenic plant *Melochia tomentosa* [15-17] as well as from fungi: 6-(3'-methylbuten-2'-yl)isatin was isolated from *Streptomyces albus* [18] and 5-(3'-methylbuten-2'-yl)isatin from *Chaetomium globosum*. [19] Isatin has also been found to be a component of coal tar. [20]

B.2 PHYSICAL PROPERTIES OF ISATIN

Isatin crystallizes from water, alcohol, or acetic acid in the form of red needles melting at 200-201°C. It is soluble in hot water, alcohol, acetic acid, and benzene but is sparingly soluble in ether. The substance is soluble in concentrated hydrochloric acid and in concentrated sulfuric acid. It dissolves in sodium or potassium hydroxide solution, forming the sodium or potassium salt of isatin. Heating the solution results in ring opening with the formation of the salt of isatic acid. Ring closure results when the solution is acidified, and isatin precipitates.

B.3 SYNTHESIS OF ISATIN

1. *The Sandmeyer methodology*

The method developed by Sandmeyer is the oldest and the most frequently used for the synthesis of isatin. It consists in the reaction of aniline with chloral hydrate and hydroxylamine hydrochloride in aqueous sodium sulfate to form an isonitrosoacetanilide, which after isolation, when treated with concentrated sulfuric acid, furnishes isatin in >75% overall yield. [21] Literature also reveals different applications [22-29] for the preparation of isatin derivatives using the Sandmeyer methodology and its disadvantages. [30-33]

2. *Use of nitroacetanilides*

Nitroacetanilides, obtained by alkaline hydrolysis of 1-arylamino-1-methylthio-2-nitroethenes are readily cyclized to isatin-3-oximes by the use of concentrated sulfuric acid or trifluoromethanesulfonic acid at room temperature; the later giving somewhat higher yields. [34]

3. *The Stolle procedure*

The most important alternative to Sandmeyer's procedure is the method of Stolle. In this method anilines are reacted with oxalyl chloride to form an intermediate chlorooxalylanilide which can be cyclized in the

presence of a Lewis acid, usually aluminum chloride or $BF_3.Et_2O$ [35], although $TiCl_4$ [36] has also been used to give the corresponding isatin. Literature also reveals different applications for the preparation of isatin derivatives using the Stolle procedure. [37-41]

4. *The Martinet isatin synthesis*

The Martinet procedure for the synthesis of indole-2,3-diones involves the reaction of an aminoaromatic compound and either an oxomalonate ester or its hydrate in the presence of an acid to yield a 3-(3-hydroxy-2-oxindole)carboxylic acid derivative which after oxidative decarboxylation yields the respective isatin. This method was applied with success for the synthesis of 5, 6-dimethoxyisatin from 4-aminoveratrole whereas the use of 2, 4-dimethoxyaniline was less successful [42] but it is readily applied to naphthylamines. [43]

5. *The Gassman procedure*

A fundamentally different and general procedure developed by Gassman is another option for the synthesis of isatins. [44, 45] This methodology consists in the formation and subsequent oxidation of an intermediate 3-methylthio-2-oxindole [46-48] to give the corresponding substituted isatins in 40-81% yield. Literature also reveals different applications for the preparation of isatin derivatives using the Gassman procedure. [49]

6. *Metalation of anilide derivatives*

A more recent method for the synthesis of isatins is based upon the directed ortho-metalation (DoM) of *N*-pivaloyl-and *N*-(t-butoxycarbonyl)-anilines. The corresponding dianions are treated with diethyl oxalate and the isatins are obtained after deprotection and cyclisation of the intermediate α-ketoesters. This method has the advantage of being regioselective for the synthesis of 4-substituted isatins from *meta*-substituted anilines where the substituent is a metalation directing group (e.g. OMe). [50] (Fig. B.1.2) Literature also reveals different applications for the preparation of isatin derivatives via metalation of anilide derivatives. [51, 52]

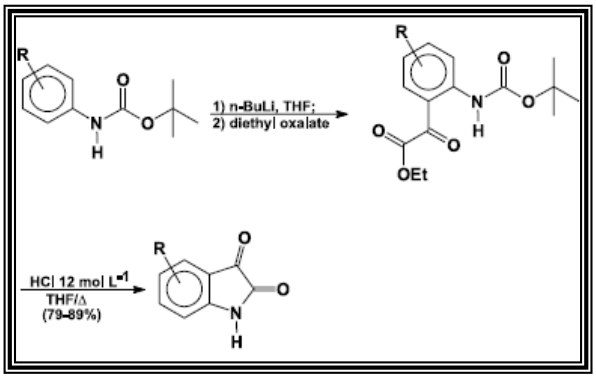


Fig. B.1.2

7. *Miscellaneous procedures*

Many other alternate procedures for the preparation of isatins are discussed in last few years. [53-98]

B.4 *N*-ALKYLATION ON ISATIN

Many methods have been devised for the *N*-alkylation of isatins. These derivatives are commonly synthesized from the reaction of the sodium salt of isatin with alkyl halides or sulphates. [99, 100] Various methods for the preparation of this salt have been reported, and include the reaction of isatin with sodium hydride, in toluene, [101] DMF, [102-133] Dioxane, [134] DMSO, [135, 136] THF. [137-142] In case of carbonates it includes, the use of potassium carbonate in DMF, [108, 133, 143-155] acetone, [156-158] acetonirile, [159, 160] chloroform, [161] while the use of cesium carbonate in acetonitrile, [160] DMF, [162] and the use of sodium carbonate in acetone. [163, 164] Strong bases like NaOH with DMF [165, 166] and methanol [166] and KOH with DMF, [167, 168] DMSO, [169] ethanol [170-173] and methanol [174-176] are also used for *N*-alkylation of isatin. TEA is also used for *N*-acylation with Benzene [103, 177] while DMF, [178] and THF [179] is used with TEA for alkylation. Sodium methoxide is used with acetonitrile, [180] methanol [181] and sodium ethoxide with ethanol. [103, 182, 183] In the later case an aldol reaction of the solvent also occurs with the C_3 carbonyl of the isatin derivative. Heating in *ortho*-dichlorobenzene results in a retro-aldol reaction and the obtention of the *N*-alkylated isatin. The use of CaH_2 in DMF has been reported [184-187] and this method was used for the synthesis of both mono and bis-*N*-alkylisatins. These

later compounds have been previously prepared using dihaloalkanes and NaH in dioxane [134] or DMF [188] or by the use of LiH. [189] Some of these alkylation methodologies were evaluated for the synthesis of isatins bearing a glycosidic residue linked to the *N*-1 position. [190] Use of KF and Al_2O_3 with acetonitrile [191] and THF [191] is also reported for *N*-alkylation of isatin. Literature also revealed uses of different bases *viz.* pyridine in benzene, [192] diisopropylethylamine in dichloromethane, [176] cuprous oxide in DMF, [193] BEMP (2-*tert*-butylimino-2-diethylamino-1, 3-dimethylperhydro-1, 3, 2-diazaphosphorine on polystyrene) in acetonitrile [194] and potassium *t*-butoxide in THF. [195]

An alternative method for preparing 1-alkylisatins consists in the reaction of isatin and alkyl halides in a benzene-chloroform/50% aq. KOH biphasic system, employing tetrabutylammonium hydrogensulfate as the phase transfer catalyst. [196]

N-propargylisatins, obtained from isatin and propargyl halides [184, 197] can be converted to *N*-acetonylisatins through hydration with Hg(II) salts in acidic media. [198]

The synthesis of 1-methylisatin by the method of Stolle, using tris (methylphenylamino) methane instead of *N*-methylaniline, leads to the desired product in low yields. [199]

The reaction of isatin with vinyl acetate in the presence of Na_2PdCl_4 yields 1-vinylisatin. [200]

On the other hand, *O*-alkylation at position 2 has been reported, along with the *N*-alkyl product, using *γ*- butyrolactone [201]or allyl bromide [202] as alkylating agents and the sodium salt of isatin. *O*-methylisatin is described as the product of the reaction of methyl iodide with the silver salt of isatin, which can be prepared from isatin and silver acetate. [203] The alkoxy group has been reported to be displaced by nucleophiles such as hydrazines. [204]

B.5 *N*-ACYLATION ON ISATIN

The synthesis of *N*-acylisatins under a variety of conditions has been described using acyl chlorides or anhydrides under reflux. The reaction may be performed without additives [205] or by using perchloric acid in benzene, triethylamine in benzene, [103] pyridine in benzene, [206] or triethylamine in chloroform [207, 208] as catalysts; or by conversion of isatin to sodium isatide using NaH in toluene under reflux and subsequent reaction with acyl chlorides. [144]

The use of diacyl chlorides such as oxalyl [209], octanedioyl or nonanedioyl chlorides [210], yields bisacylisatins. Attempts to use 2, 2-dimethylmalonyl chloride to furnish 2, 2-dimethylmalonyl-bis-isatin failed, and led instead to an unusual tricyclic compound which was characterized by spectroscopic methods and by X-ray diffraction. [211] (Fig. B.1.3)

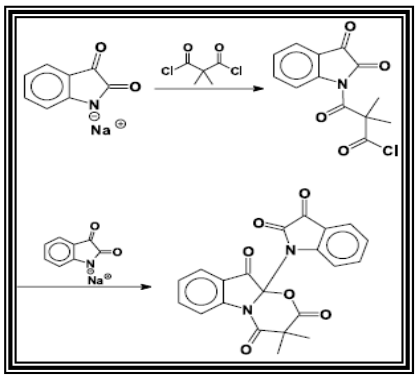


Fig. B.1.3

Other complex products have been obtained from the reaction of isatin and acetic anhydride in the presence of pyridine. [212] (Fig. B.1.4)

Similarly, dimers may be formed in the acetylation of indolylglyoxalates with acetic anhydride in pyridine. [213] (Fig. B.1.5)

Many authors [214-230] have reported *N*-acylation or *N*-acetylation on isatin.

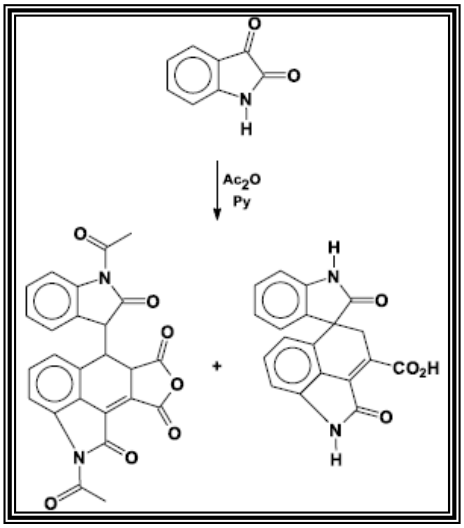


Fig. B.1.4

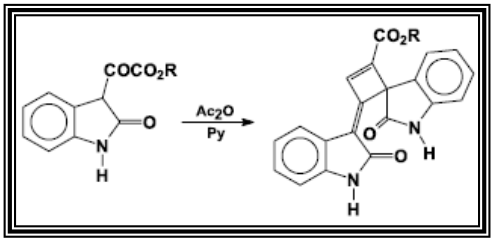


Fig. B.1.5

B.6 MANNICH REACTION ON ISATIN

The Mannich reaction consists in the condensation of ammonia or a primary or secondary amine, usually as the hydrochloride, with formaldehyde and a compound containing at least one hydrogen atom of pronounced reactivity. The essential feature of the reaction is the replacement of the active hydrogen atom by an aminomethyl or substituted aminomethyl group. The Mannich reaction is readily applied to isatins.

Varma and Nobles [231] synthesized a series of Isatin-*N*-Mannich bases derived from isatin (Fig. B.1.6), 5-methyl and 5-bromoisatins as the active hydrogen component and dimethylamine, diethylamine, morpholine, piperidine, *N*-ethylcyclohexylamine, 3-azabicyclo [3.2.2] nonane and 3-aza-bicyclo [3.2.1] octane as the secondary amine moiety.

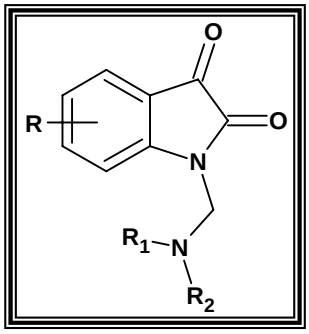


Fig. B.1.6

R = 5-H, 5-CH_3, 5-Br and R_1 & R_2 = Different Secondary Amines

The products of Mannich reaction, the *N*-aminomethylisatins (Mannich bases), can also be obtained from the *N*-hydroxymethyl derivatives by reaction with an amine [232] or by reaction with acetyl chloride to yield *N*-chloromethylisatin which can be further treated with potassium phthalimide or an alcohol to give the corresponding *N*-phthalimidomethyl or *N*-alkoxymethyl isatins. [233] The Mannich reaction can also be performed with isatin derivatives, such as isatin-3-hydrazones [234] and isatin-3-thiosemicarbazones. [235]

Jozef [236] prepared a series of twenty Mannich bases by treating cyclic imides and hydrazides with formaldehyde and secondary amines. Thus, phthalic hydrazide yielded (1) (R = R^1 = cyclohexyl, CH_2CH_2OH, Ph; R = Ph, R^1 = CH_3). (2) (R = R^1 = cyclohexyl, Ph, Et, CH_2CH_2OH), (3) (R = R^1 = Ph, cyclohexyl, CH_2CH $(CH_3)_2$, $CH_2CHOHCH_3$; R = Ph, R^1 = CH_3), (4) (R = R^1 = $CH_2CHOHCH_3$; R = Ph, R^1 = cyclohexyl), and (5) (R = R^1 = Ph, CH_2CH $(CH_3)_2$, $CH_2CHOHCH_3$; R = Ph, R^1 = cyclohexyl, R = Ph, R^1 = $PhCH_2$) were similarly prepared bis(aminomethyl) compounds were obtained from maleic and phthalic hydrazides and from isatin when the piperazine was used. (Fig. B.1.7)

Varma and Khan [237] treated (hydroxymethyl) indolinone (1) with 4-chloroaniline in refluxing ethanol gave the indolinone (2) (R = H). Then they carried out Mannich reaction of (2) to give (2) (R = piperidinomethyl, morpholinomethyl). Treatment of (1) with 4-nitroaniline, 4-methylaniline, or methyl-4aminobenzoate gave indolinone (3). (Fig. B.1.8)

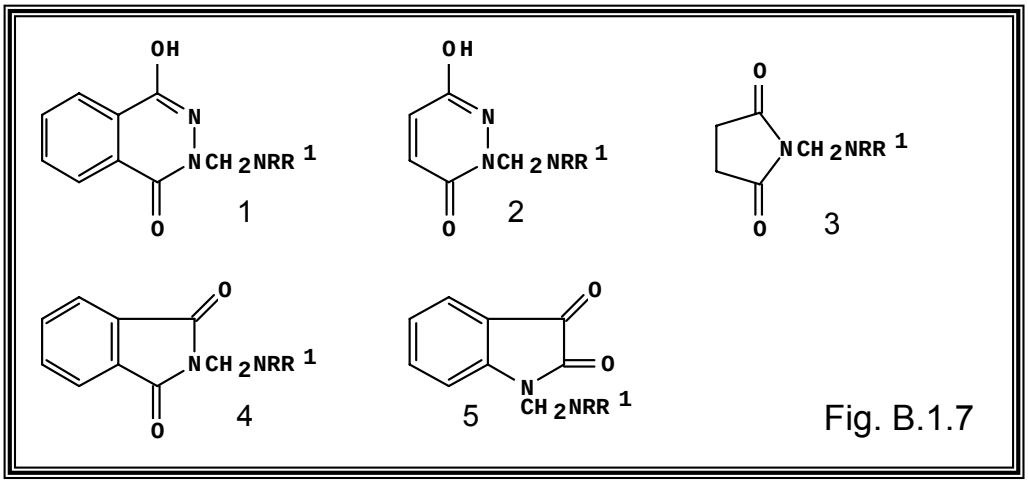


Fig. B.1.7

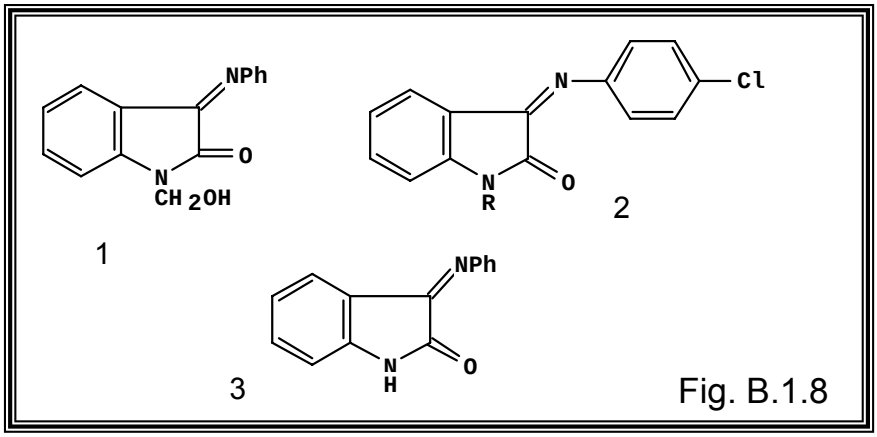


Fig. B.1.8

They [238] further carried out condensation of (1) (R = CH_2OH, R^1 = H) with 4-iodoaniline gave (1) (R = H, R^1 = p-IC_6H_4) and not the expected product (1) (R = p-$IC_6H_4NHCH_2$, R^1 = H). (Fig. B.1.9)

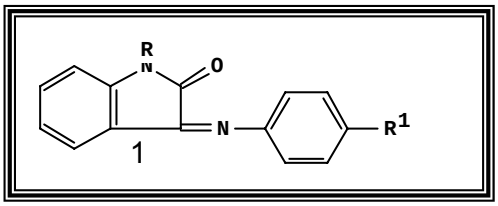


Fig. B.1.9

Joshi *et. al.* [239] prepared fluorotriazinoindoles (1) (R = 7-F, 8-F; N(R^1)2 = Et_2N, piperidino, pyrrolidino, morpholino) and fluorodiazoindolones (2) (R^2 = Ph, 4-FC_6H_4, 4-$Me_2NC_6H_4$, 4-ClC_6H_4, 2-furyl, 4-$O_2NC_6H_4$) from fluoroindolediones (3) (R = 5-F, 6-F). (Fig. B.1.10)

The same group [240] also prepared aminoacetylindolediones (1) (R = 5-F, 6-F; NR^1R^2 = NMe_2, NEt_2, morpholino, piperidino) by cyclizing fluoroisonitrosoacetanilides with acid, chloroacetylating the fluoroindolediones, and aminating the chloroacetyl derivatives. (2) [R^3 = 4-F, 4-Cl, 4-NO_2, 2, 4-$(NO_2)_2$] were obtained by Mannich reaction of the fluoroindolediones with morpholine and reaction with phenyl hydrazines. ($R^3C_6H_4NHNH_2$) (Fig. B.1.11)

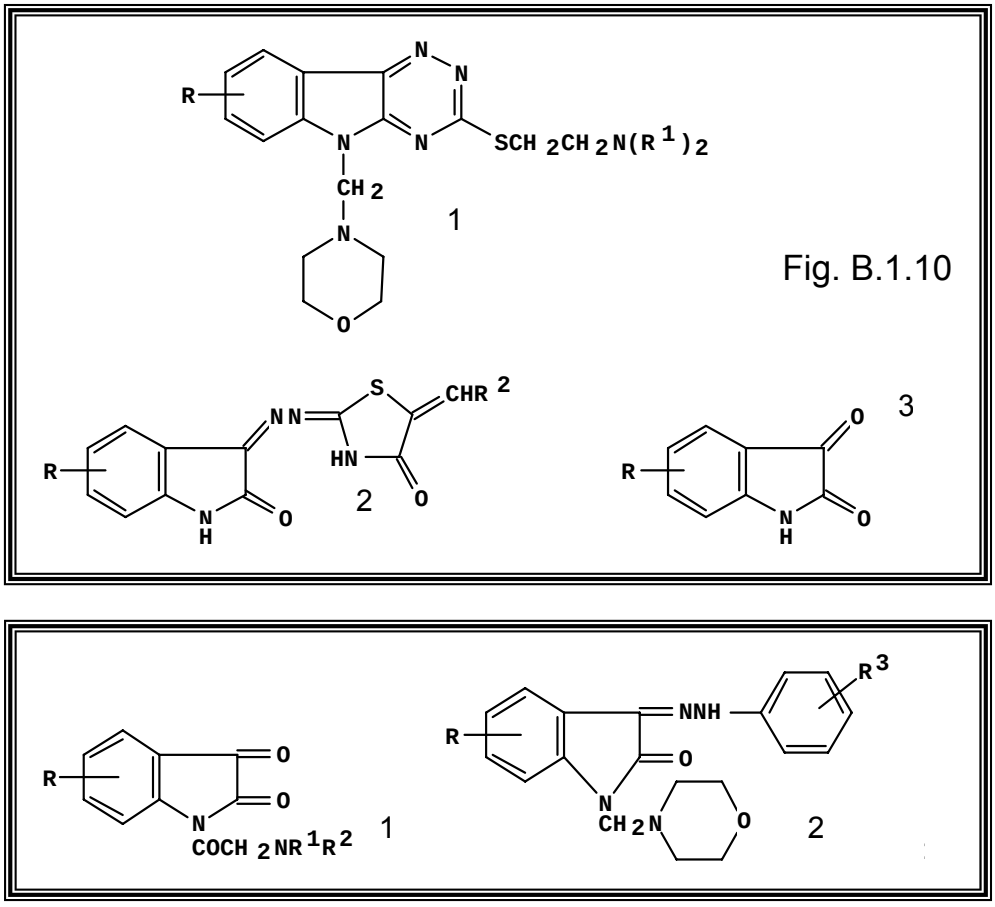


Fig. B.1.11

They [241] further reported that Mannich reaction of indolediones (1) (R = H; R^1 = 4-CF_3, 5-F, 6-F; X = O) and amines (R^2H = morpholine, piperidine) gave 46-73 % (1) (R = CH_2R^2). Treating (1) (R = H; R^1 = 4-CF_3, 5-F, 6-F; X = O) with R^3NH_2 (R^3 = 4-FC_6H_4, 3-$CF_3C_6H_4$) gave 71-82 % (1) (X = NR^3), which underwent Mannich reaction with R^2H to give 50-64 % (1) (R = CH_2R^2). Refluxing thiosemicarbazide with (1) (R =H, X = O) in K_2CO_3-H_2O gave 78-82 % 1, 2, 3-triazino [5, 6-b] indole-3-thiones (2). Successive treatment of (2) with

NaOH and $R^2CH_2CH_2Cl.HCl$ gave 61-73 % (3) (R^2 = morpholino, piperidino, pyrrolidino). (Fig. B.1.12)

Collino and Volpe [242] prepared Mannich bases from benzimidazoles, benzotriazoles, indolones, or benzoxazolones and *N*-phenylpiperazine, 4-anilinopiperidine, 4-benzylpiperidine, or *N*-bezylpiperazine.

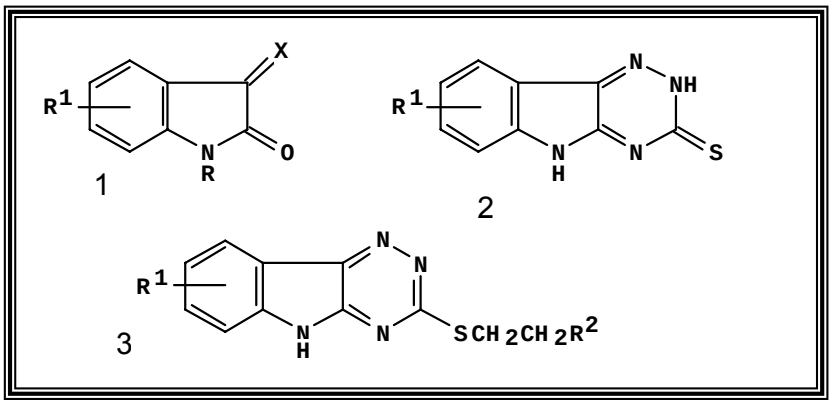
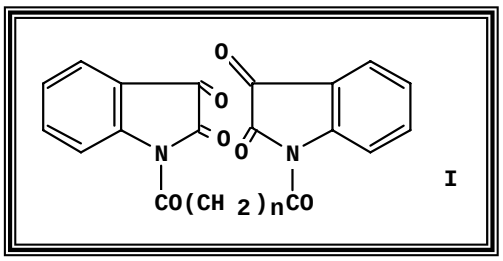


Fig. B.1.12

The same authors [243] further prepared Mannich bases of *N* heterocycles or aromatic amines with trimethylenedipiperidine, dipiperidine, and hydroxyethyldipiperidine as well as the isatin derivatives (where, n = 6, 7). (Fig. B.1.13)



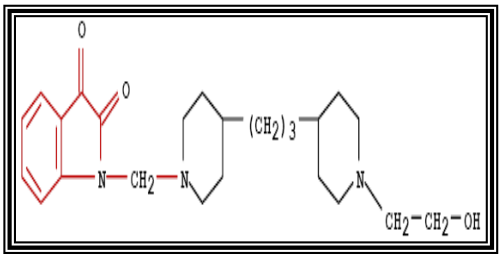


Fig. B.1.13

Jancevska-Nikolovska [244] converted the hexylbenzene into 4-hexylaniline which underwent cyclization reactions to give acridines, benzacridines, isatins, carbazoles, indoles, benzindoles, quinolines, and benzoquinolines. (all substituted with the hexyl group) Further they carried out Mannich reaction to give *N*-substituted derivatives. (Fig. B.1.14)

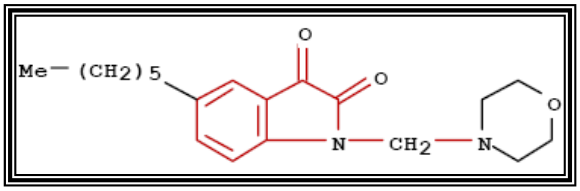


Fig. B.1.14

Popp and Rajopadhye [245] prepared the title compounds by the cyclocondensation of thiolactic acid with isatin-3-imines. 5'-methyl-3'-phenyl-spiro [3*H*-indole-3, 2'-thiazolidine]-2, 4' (1*H*)-dione has been subjected to the Mannich condensation to give 1-substituted derivatives. (Fig. B.1.15)

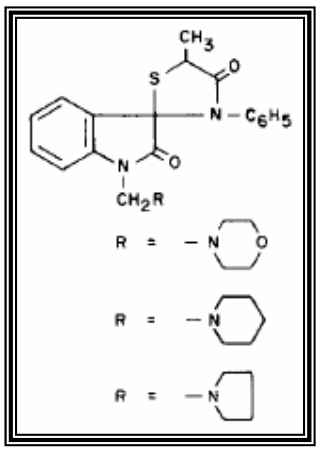


Fig. B.1.15

Further they [246] synthesized Mannich base of 3'-(4-chlorophenyl)-5, 5'-dimethyl-4'*H*-spiro[indole-3, 2'-[1, 3]thiazolidine]-2, 4'(1*H*)-dione using formaldehyde and morpholine along with differently substituted isatin derivatives. (Fig. B.1.16)

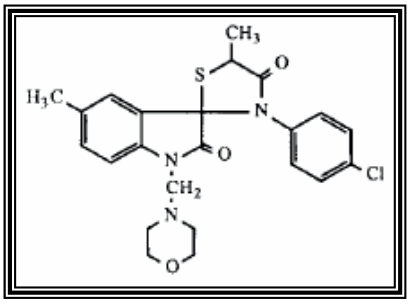


Fig. B.1.16

Varma and Singh [247] prepared differently substiuted isatins and then they reported Mannich bases of afore mentioned isatins. (Fig. B.1.17)

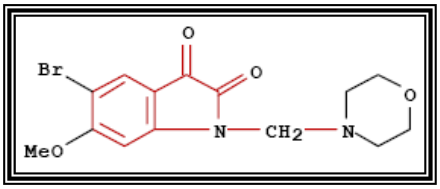


Fig. B.1.17

Pandeya *et. al.* [248] synthesized Mannich bases of norfloxacin by reacting them with formaldehyde and several isatin derivatives. The starting materials 3-[4-sulfadiazinimino] isatin, 3-[4(-sulfadoximino] isatin and 3-[4'-amino, 5'-(3'', 4'', 5''-trimethoxybenzyl)pyrimidin-2'-yl]imino isatin and their 5-substituted derivatives were prepared by reacting isatin and its derivatives with sulfadiazine, sulfadoxine and trimethoprim in the presence of glacial acetic acid. Mannich bases of norfloxacin were prepared by condensing the active hydrogen atom of isatin and the secondary amino function (piperazino moiety) of norfloxacin. (Fig. B.1.18)

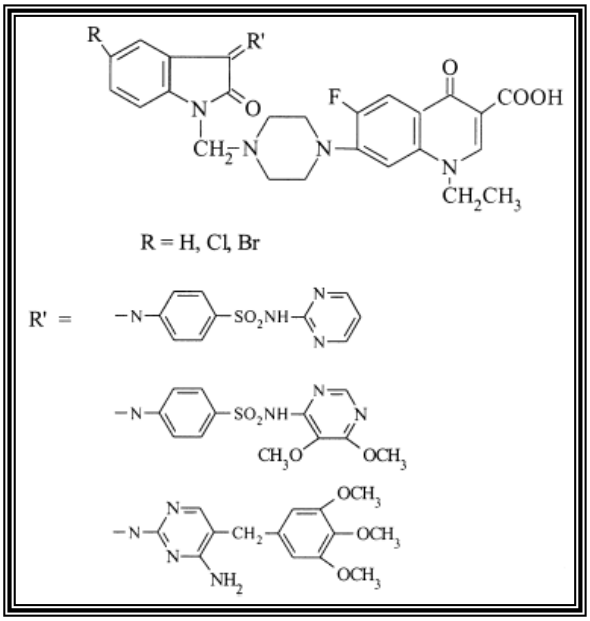


Fig. B.1.18

Korepin *et. al.* [249] reported Mannich reactions of amino alchohols 3-aminopropan-1-ol or 2-aminoethanol with formaldehyde and C-H or N-H acids gave *N*-substituted tetrahydro-1, 3-oxazines or oxazolidines. Thus, reaction

of 3-aminopropanol (1) with formaldehyde and 1, 1-dinitroethane or 2-bromo-2, 2-dinitroethanol gave the 1, 3-oxazines (2) (R = Me, Br). Similar reaction of (1) with oxalamide and succinamide gave the bis (oxazinyl) diamides (3) (X = bond, CH_2CH_2) and reaction of 2-aminoethanol with formaldehyde and benzotriazole gave the oxazolylmethylbenzotriazole. (Fig. B.1.19)

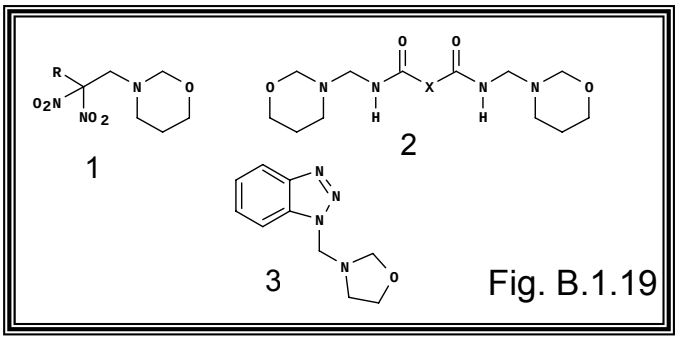


Fig. B.1.19

Furthermore they [250] reported that the reactions of RH (RH = isatin, benzotriazole, and succinimide) with formaldehyde and methylamine yield monoamines ($RCH_2N(Me)CH_2R$) and methylenediamines. ($RCH_2N(Me)CH_2N(Me)CH_2R$) The use of ethylenediamine as the amino component affords *N*, *N*'-disubstituted imidazolidines, while the reactions with 3-aminopropanol give *N*-substituted tetrahydro-1, 3-oxazines. RCH_2NBui_2 was obtained from succinimide, formaldehyde, and diisobutylamine. Nitrosative cleavage of the amines obtained was studied: monoamines and methylenediamines give *N*-nitrosoamines. ($RCH_2N(NO)Me$) RCH_2NBui_2 affords diisobutylnitrosamine, while imidazolidines transform into dinitroso compounds. ($RCH_2N(NO)CH_2CH_2N(NO)CH_2R$) (Fig. B.1.20)

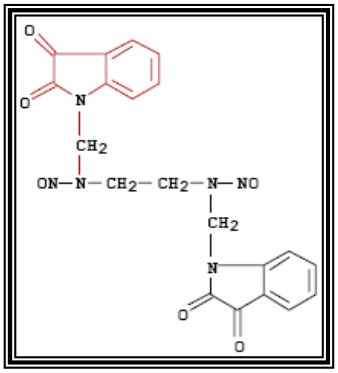


Fig. B.1.20

Mesropyan *et. al.* [251] synthesized new *N*-derivatives of isatin by treating ethyl- chloroacetate, *N*-(2-chloroethyl) morpholine, and 1, 4-bis(chloromethyl)benzene with isatin sodium salt. *N*-derivatives of isatin and 5-bromoisatin were also prepared by Mannich reaction. (Fig. B.1.21)

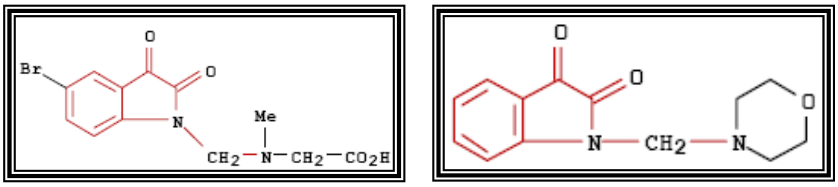


Fig. B.1.21

Mogilaiah *et. al.* [252] disclosed a conventional method for the preparation of the Mannich base of 3-[3-(p-chlorophenyl)-1, 8-naphthyridin-2-ylhydrazono]-2-indolinones using formaldehyde and piperidine. The starting material was prepared by the condensation of 2-hydrazino-3-(p-chlorophenyl)-1, 8-naphthyridine and 3-phenylimino-2-indolinones. (Fig. B.1.22)

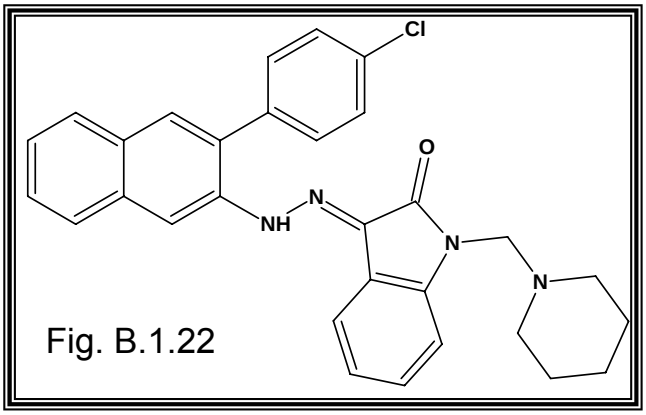


Fig. B.1.22

Yogeeswari *et. al.* [253] synthesized Mannich bases of gatifloxacin by reacting them with formaldehyde and several isatin derivatives. (Fig. B.1.23)

Sriram *et. al.* [254] reported the synthesis of various aminopyrimidinimino isatin derivatives which was achieved in two steps. First step consists condensation of 5-bromo isatin and 5-(3, 4, 5-trimethoxybenzyl) pyrimidine-2,4-diamine followed by Mannich reaction using formaldehyde and various secondary amines (R'). (Fig. B.1.24)

A new class of 4-aminoquinoline derivatives based on the natural product isatin scaffold were designed and synthesized using Mannich reaction on isatin and isatin thiosemicarbozones by Chiyanzu *et. al.* [215] (Fig. B.1.25)

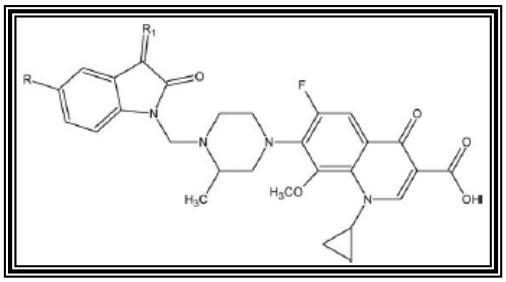
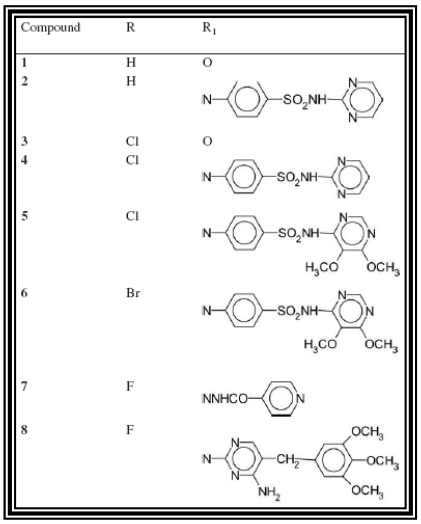


Fig. B.1.23



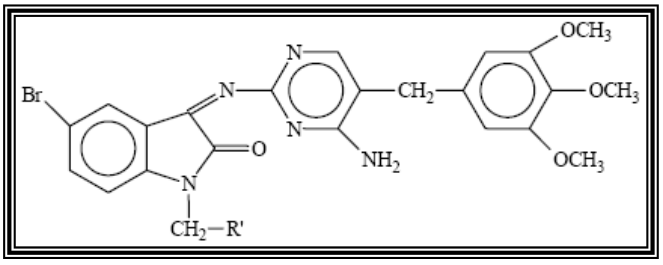


Fig. B.1.24

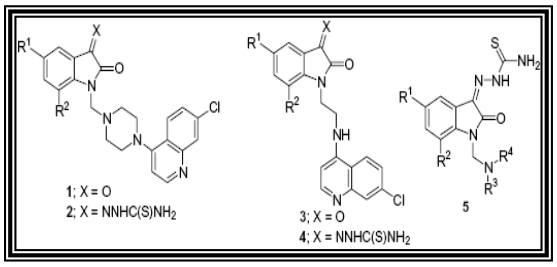


Fig. B.1.25

R^1	R^2	$(R^3)R^4$	X
H	H	—	O
Me	Me	—	O
Cl	H	—	O
F	H	—	O
Me	H	—	O
I	H	—	O
Br	H	—	O
H	H	—	$NNHC(S)NH_2$
F	H	—	$NNHC(S)NH_2$
Cl	H	—	$NNHC(S)NH_2$
Br	H	—	$NNHC(S)NH_2$
Me	H	—	$NNHC(S)NH_2$
H	H	NEt_2	$NNHC(S)NH_2$
H	H		$NNHC(S)NH_2$
H	H		$NNHC(S)NH_2$
H	H		$NNHC(S)NH_2$
H	H		$NNHC(S)NH_2$
H	H		$NNHC(S)NH_2$

Sriram *et. al.* [255] synthesized some 7-substituted ciprofloxacin derivatives by Mannich reaction using ciprofloxacin, formaldehyde and differently substituted isatin and its derivatives. (Fig. B.1.26)

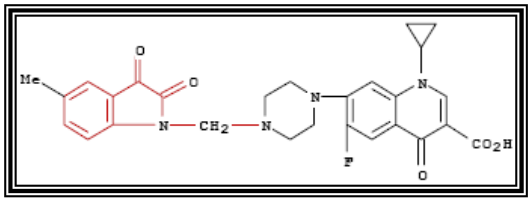


Fig. B.1.26

Mohamed *et. al.* [256] prepared *N*-Mannich bases of phthalimide, isatin, 5-fluoroisatin, 5-chloroisatin, diphenylamine and succinimide using formaldehyde and 8-Hydroxyquinoline to give title compounds. (Fig. B.1.27)

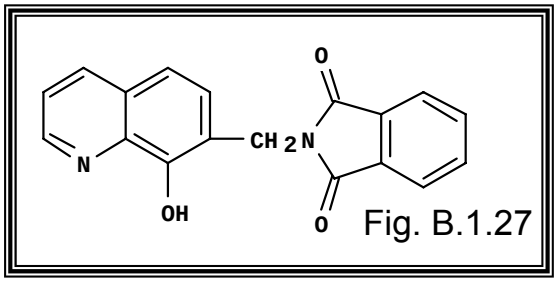


Fig. B.1.27

Ravichandran *et. al.* [257] prepared a series of 2, 3-dihydro-2-oxo-1, 3-disubstituted indoles by the reaction of 2, 3-dihydro-2-oxo-3-substituted indoles with 2-[(2, 6-dichlorophenyl) amino] phenylacetic acid in the presence of formaldehyde. The title compounds were synthesized in two steps. First step consists condensation of substituted amine and isatin while second step involves Mannich reaction using formaldehyde and 2-[(2, 6-dichlorophenyl) amino] phenylacetic acid. (Fig. B.1.28)

Where, R = 4-chloroaniline, 4-amino benzene sulphonamide, 4-amino benzoic acid, 2-amino benzoic acid, Phenyl hydrazine.

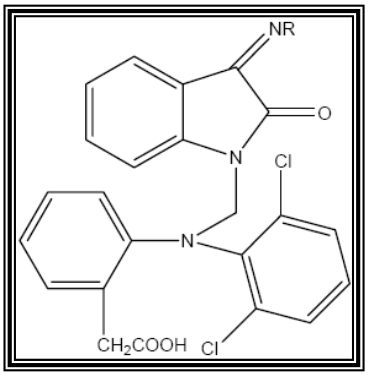


Fig. B.1.28

Esmaeili *et. al.* [258] prepared *N*-Mannich bases of isatin by refluxing isatin, formaldehyde and secondary amine in tetrahydrofuran.

Tiwari *et. al.* [259] synthesized 5-substituted-3-{4'-(3''-chlorobenzyloxy)-benzoyl hydrazono}-2-indolinones by the condensation of 4-(3'-chlorobenzyloxy)-benzoylhydrazine and 5-substituted isatins. Mannich reaction in the presence of formaldehyde and heterocyclic secondary amines on indolinones furnished 1-aminomethyl-5-substituted-3-{4'-(3''-chlorobenzyloxy)-benzoyl hydrazono}-2-indolinones. (Fig. B.1.29)

Where, R_1 = H, Me, Br, Cl

R_2 = Morpholine, Piperidine, Pyrrolidine, N-Me-Piperazine

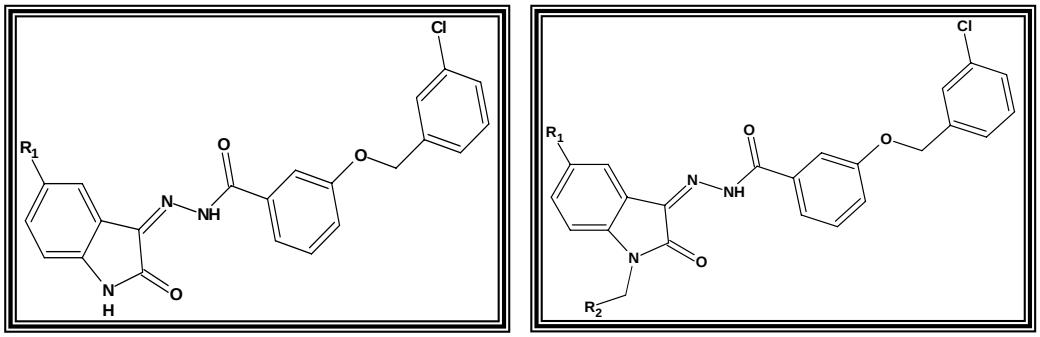


Fig. B.1.29

Bekircan and Bektas [260] reported preparation of Schiff base of substituted isatins and substituted 4-amino-4, 5-dihydro-1*H*-1, 2, 4-triazole-5-ones which afterwards underwent Mannich reaction using piperidine and formaldehyde to give title compounds. (Fig. B.1.30) (R = 2-fluoro aniline and 4-fluoro aniline, X = H, Cl)

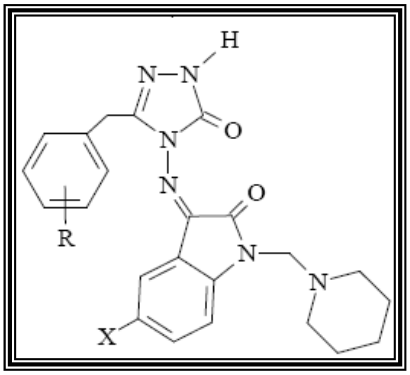


Fig. B.1.30

B.7 BIOLOGICAL ACTIVITIES ASSOCIATED WITH ISATINS

Several isatin derivatives are showing excellent pharmacological profiles. Following biological activities are reported for isatins developed by various research groups and explored.

1 CNS depressant activities [261-263]
2 Anticonvulsant activity [264-295]
3 Anxiogenic and other CNS activities [296-302]
4 Antimicrobial activity [303-329]
5 Anticancer activity [330-340]
6 Antiplasmodial activity [341, 342]
7 Antitubercular activity [343-345]
8 Inhibitor of glucose, aminoacid uptake [346, 347]
9 Isatin derivatives as serine protease inhibitors [348]
10 Isatin derivatives as SARS (Severe Acute Respiratory Syndrome) coronavirus 3CL protease inhibitors [349]
11 Isatin derivatives as oxytosin receptor antagonists [350]

12 Isatin analogs with potent galanin GAL_3 receptor binding affinity [351]

13 Isatin derivatives as ADAMTS-5 (Aggrecanase-2) inhibitors [352]

14 Antileshmanial activity [353]

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CHAPTER – 3

PREPARATION OF SMALL LIBRARY OF POTENTIAL ANTICANCER AGENTS: SCHIFF BASES FROM ISATIN CORE STRUCTURE

3.1 AIM OF CURRENT WORK

Isatin has been known for about 150 years and their derivatives constitute a class of biologically active heterocycles which have been found associated with antiviral, antibacterial, anthelmintic, amoebicidal, antifungal, anti-HIV, anticonvulsant, antileukemic, antifertility, herbicidal, antiinflammatory and CNS depressant derivatives. In addition to these cysticidal and hypotensive responses have also been reported in certain isatin derivatives.

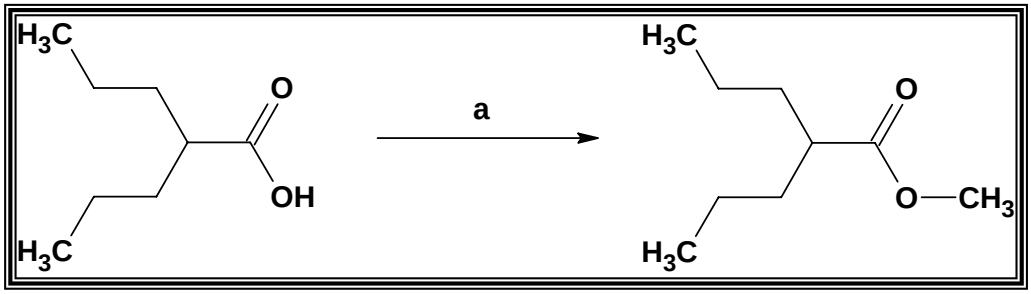
A large number of *C*-Mannich bases have been prepared and tested for analgesic, antispasmodic, anesthetic and antibacterial activity. Certain Mannich bases with complex amine moieties have shown pronounced antibacterial activity. Though considerable work has been reported on the synthesis and pharmacological activity of Mannich bases derived from compounds containing acidic hydrogen on carbon, only few examples of the Mannich reaction of compounds containing acidic hydrogen on a nitrogen atom are known and even fewer have been evaluated for their pharmacological action.

Schiff bases and Mannich bases of isatin are known to possess a wide range of pharmacological properties including antibacterial, anti-HIV, anticonvulsant, antifungal and antiviral activity. This led us to synthesize some of the hitherto unreported *N*-Mannich bases of isatin along with their Schiff bases and to report their anticancer activity. Total reported synthesis is consisting of two steps where first step includes synthesis of isatin *N*-Mannich bases using secondary amines *viz.* morpholine, piperidine, 1-methyl piperazine, 1-ethyl piperazine and 1-benzyl piperazine while second step includes Schiff bases of these *N*-Mannich bases using 2-propylpentanohydrazide and 2-amino-6-methoxy benzothiazole. Looking to the diversified biological profile shown by benzothiazoles one more scheme has been included in this chapter where Schiff bases of *N*-acetyl isatin have been prepared.

Preparation of unreported and novel isatin derivatives and their biological evaluation was the rational behind the built up of this chapter.

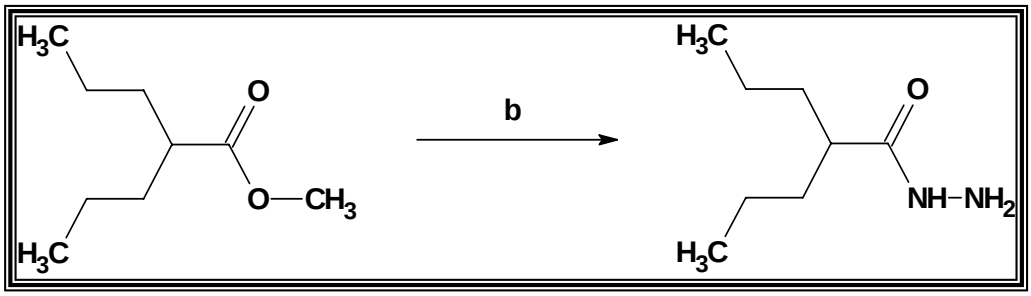
3.2 REACTION SCHEMES

3.2.1 PREPARATION OF METHYL-2-PROPYLPENTANOATE



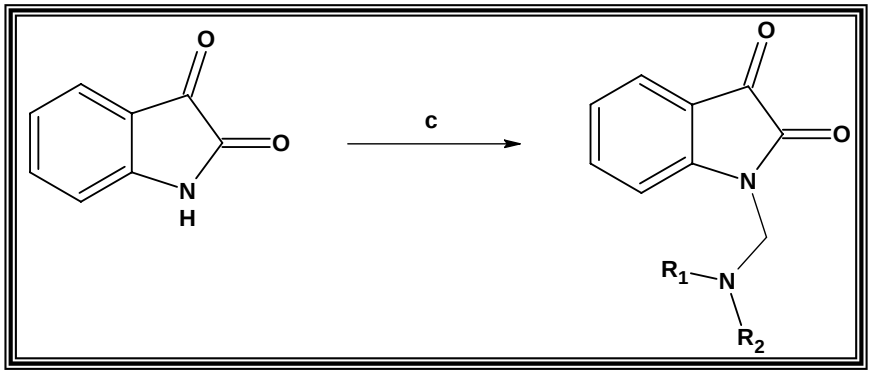
Reagents / Reaction Condition (a): Methanol, Con. H_2SO_4 / Reflux, 12-14 hours.

3.2.2 PREPARATION OF 2-PROPYLPENTANOHYDRAZIDE



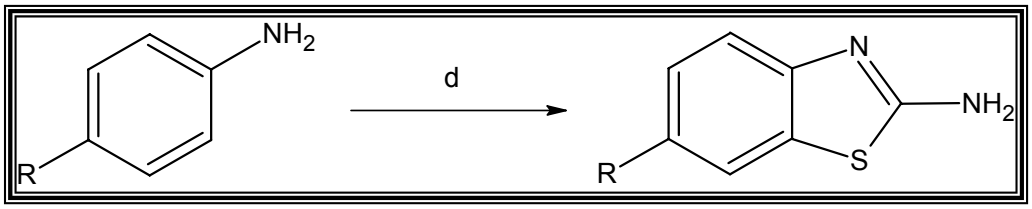
Reagents / Reaction Condition (b): Hydrazine hydrate / Reflux, 12-14 hours.

3.2.3 PREPARATION OF ISATIN-*N*-MANNICH BASES



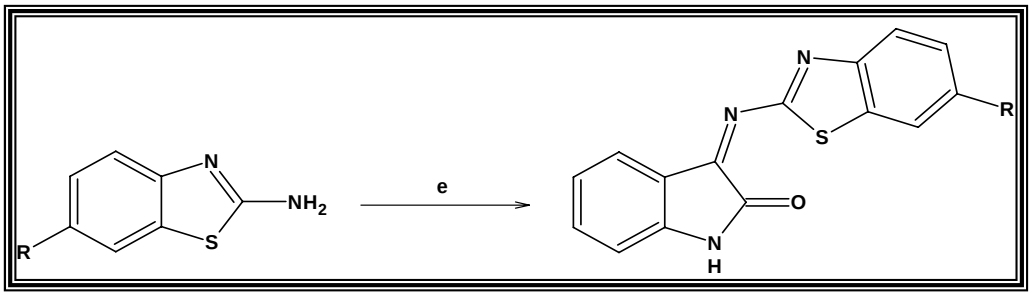
Reagents / Reaction Condition (c): Isatin, 50% Ethanol, Formaldehyde (37-41%) / 0-5°C.

3.2.4 PREPARATION OF 2-AMINO BENZOTHIAZOLE



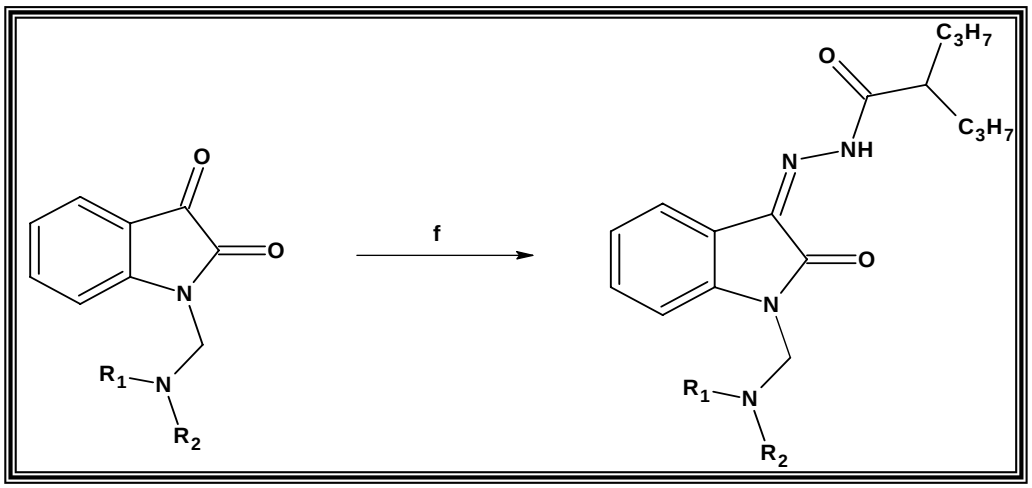
Reagents / Reaction Condition (d): KSCN, Br_2, Glacial acetic acid / 0-5°C.

3.2.5 PREPARATION OF 3-((UN) SUBSTITUTED 1, 3-BENZOTHIAZOL-2-YL IMINO)-1, 3-DIHYDRO-2*H*-INDOL-2-ONE



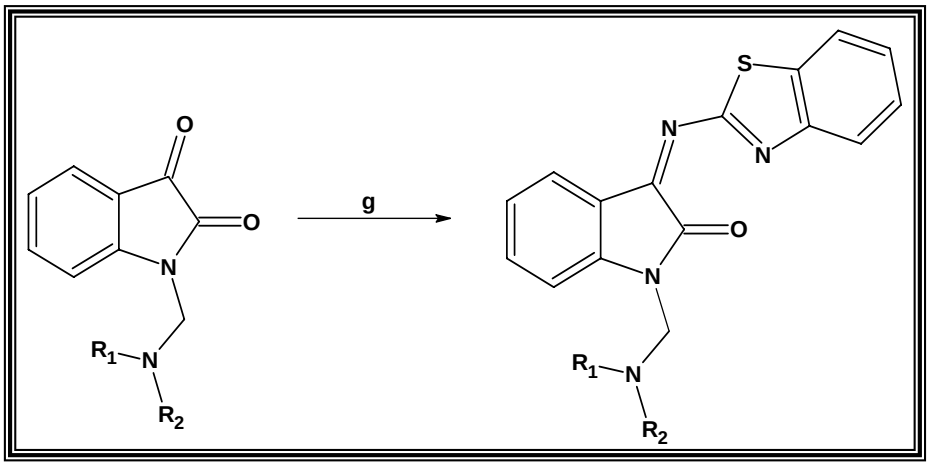
Reagents / Reaction Condition (e): Isatin, Ethanol, Con. H_2SO_4 / Reflux, 4 hours.

3.2.6 SCHEME - 1



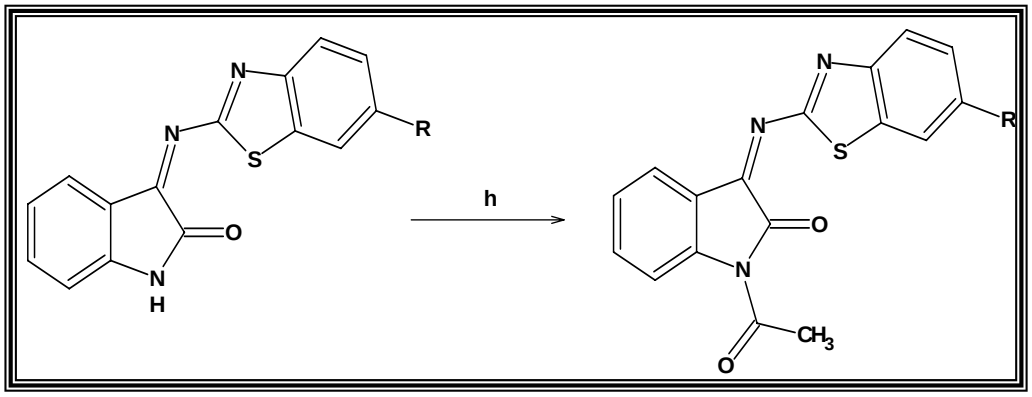
Reagents / Reaction Condition (f): 2-propylpentanohydrazide, Glacial acetic acid, Ethanol / Reflux, 5 hours.

3.2.7 SCHEME - 2



Reagents / Reaction Condition (g): 2-amino benzothiazole, Con. H_2SO_4, Ethanol / Reflux, 4 hours.

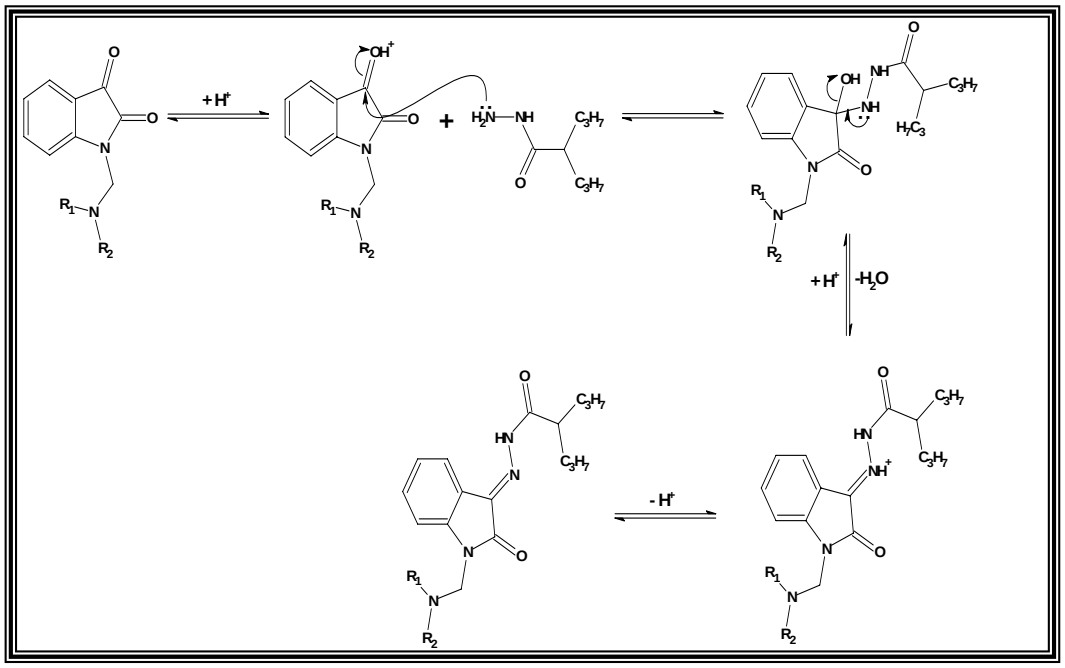
3.2.8 SCHEME - 3



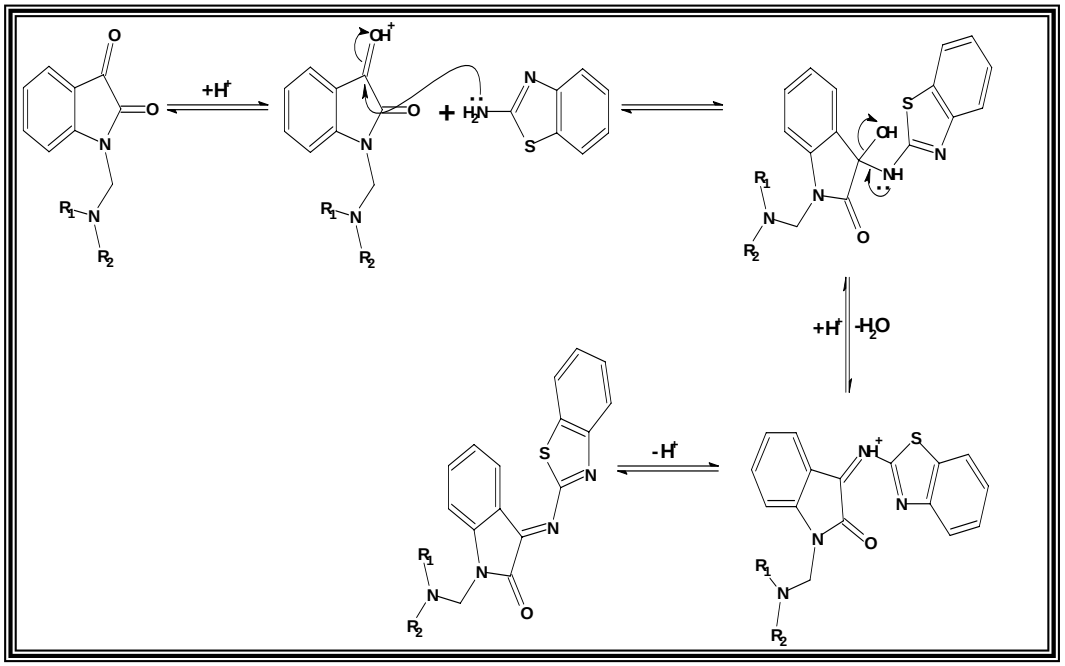
Reagents / Reaction Condition (h): Acetic anhydride / Reflux, 5 hours.

3.3 REACTION MECHANISM

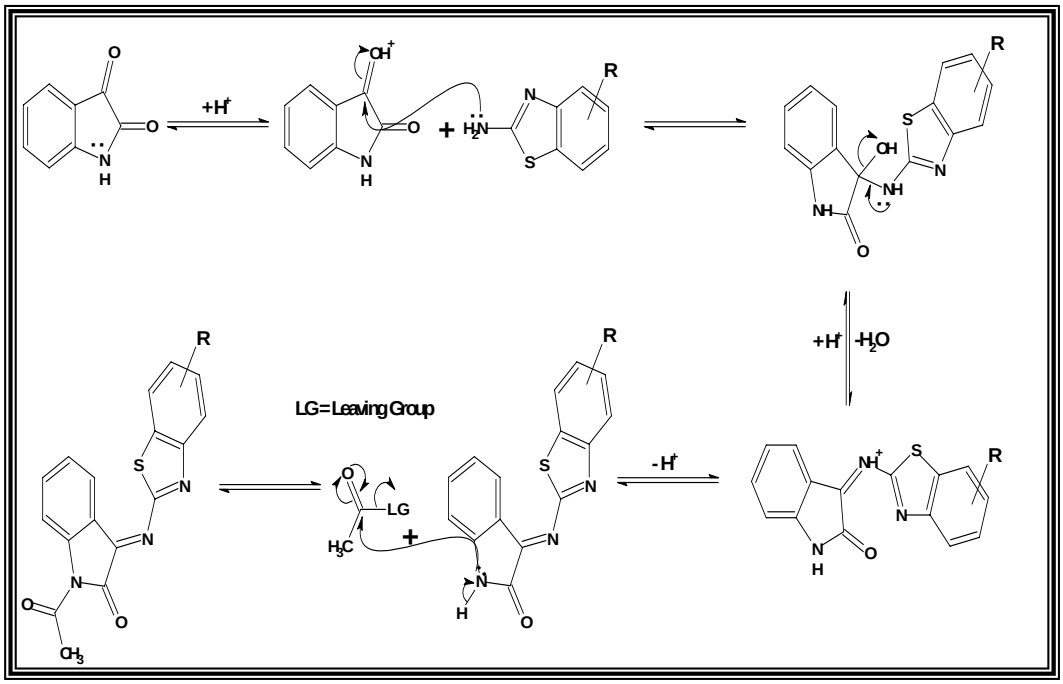
3.3.1 SCHEME - 1



3.3.2 SCHEME - 2



3.3.3 SCHEME - 3



3.4 EXPERIMENTAL

3.4.1 PREPARATION OF METHYL 2-PROPYLPENTANOATE

0.01 mole of 2-propyl pentanoic acid was charged into 250 ml round bottom flask. 15 ml of methanol was added into above flask. 3-4 drops of Con. Sulphuric acid was added as a catalyst. The reaction mixture was refluxed for 12-14 hours on water bath. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using hexane : ethyl acetate (4 : 6) as a mobile phase. After the reaction to be completed excess of methanol was removed under reduced pressure. The separated product was extracted using ethyl acetate (30 ml X 3), the combined organic layer was washed using 5% sodium bicarbonate solution (20 ml X 2) followed by water (20 ml X 2). The organic layer was dried on anhydrous sodium sulphate and the solvent was removed under reduced pressure to acquire the product in a viscous liquid form. Yield - 90 %, B. P. - 190-195°C.

3.4.2 PREPARATION OF 2-PROPYLPENTANOHYDRAZIDE

0.01 mole of methyl 2-propylpentanoate was charged into 250 ml round bottom flask. 15 ml of hydrazine hydrate was added into above flask. The reaction mixture was refluxed on water bath for 12-14 hours. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using hexane : ethyl acetate (4 : 6) as a mobile phase. After the reaction to be completed, the mixture was cooled to room temperature to give 2-propylpentanohydrazide as a white coloured shining fluffy product. Yield - 60 %, M. P. - 120-122°C.

3.4.3 PREPARATION OF ISATIN-*N*-MANNICH BASES

It was prepared according to the method described by Varma and Nobles. [a] To slurry consisting of 0.04 mole of isatin, 5 ml 50% ethanol and 5 ml formaldehyde solution (37-41%) was added 0.04 mole of the desired

[a] R. S. Varma and W. L. Nobles; *J. Het. Chem.*, **1966**, *3(4)*, 462.

secondary amine, drop wise, with cooling and shaking. The reaction mixture was allowed to stand at room temperature for one hour with occasional shaking after which it was warmed on a steam bath for 15 minutes. At the end of this period the contents were cooled and the product thus separated was filtered and recrystallized from ethanol.

Code	Substitution R_1 & R_2	MF	MW (g/m)	MP (°C)	% Yield
IMB-01	Morpholine	$C_{13}H_{14}N_2O_3$	246	202-204 [b]	70
IMB-02	Piperidine	$C_{14}H_{16}N_2O_2$	244	146-148 [c]	65
IMB-03	1-Methyl Piperazine	$C_{14}H_{17}N_3O_2$	259	178-180	67
IMB-04	1-Ethyl Piperazine	$C_{15}H_{19}N_3O_2$	273	192-194	63
IMB-05	1-Benzyl Piperazine	$C_{20}H_{21}N_3O_2$	335	128-130 [d]	60

3.4.4 PREPARATION OF 2-AMINO BENZOTHIAZOLES

It was prepared according to the method described by Stuckwisch. [e] To a solution of 0.2 mole of an appropriate amine and 0.8 mole of potassium thiocyanate in 360 ml of glacial acetic acid was added drop wise, with stirring, 0.2 mole of bromine dissolved in 150 ml of glacial acetic acid while the temperature was kept below 35 °C. After all the bromine solution had been added, the mixture was stirred for ten hours and was then filtered and the residue washed with water. The combined filtrate and washings were neutralized with ammonium hydroxide. The precipitate was collected on a filter and dried. This material was pure enough for subsequent reactions. Further purification was most readily carried out by recrystallization from a mixture composed of equal volumes of concentrated hydrochloric acid and 95% ethanol. The hydrochloride thus obtained was dissolved in water and the free base was precipitated with sodium carbonate. The recovery of 2-amino benzothiazole was nearly quantitative.

[b] Reported : 203-205°C; A. A. Esmaeili, S. Amini and A. Bodaghi; *Synlett*, **2007**, *9*, 1452.

[c] Reported : 147-148°C; R. S. Varma and I. A. Khan; *Nat. Acad. Sci. Lett.*, **1979**, *2(4)*, 137.

[d] Reported : 128-129°C; F. Collino and S. Volpe; *Boll. Chim. Farmace.*, **1982**, *121(5)*, 221.

[e] C. G. Stuckwisch; *J. Am. Chem. Soc.*, **1949**, *71*, 3417.

Code	Substitution R	MF	MW (g/m)	MP (°C)	% Yield
ABT-01	4-H	$C_7H_6N_2S$	150	126-128	79
ABT-02	4-OCH_3	$C_8H_8N_2OS$	180	166-168	83
ABT-03	4-Cl	$C_7H_5ClN_2S$	184	200-202	80
ABT-04	4-F	$C_7H_5FN_2S$	168	182-184	81
ABT-05	4-NO_2	$C_7H_5N_3O_2S$	195	248-250	77

3.4.5 PREPARATION OF 3-((UN) SUBSTITUTED 1, 3-BENZOTHIAZOL-2-YL IMINO)-1, 3-DIHYDRO-2*H*-INDOL-2-ONE

It was prepared according to the method reported by Chohan *et. al.* [f] To a stirred solution of 0.01 mole of an appropriately substituted 2-amino benzothiazole in 50 ml warm ethanol was added 0.01 mole 1*H*-indole-2, 3-dione in 60 ml ethanol. Then 2–3 drops of conc. sulphuric acid were added and the reaction mixture was refluxed for 4 hours. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using hexane : ethyl acetate (6 : 4) as a mobile phase. After the reaction to be completed, the flask was cooled to afford a solid product. The solid residue was filtered, washed with cold ethanol, then with ether and dried. Recrystallization was carried out from hot ethanol.

Code No.	Substitution R	MF	MW (g/m)	MP (°C)	% Yield
DNJ-1500-A	4-H	$C_{15}H_9N_3OS$	279	164-166	55
DNJ-1500-B	4-OCH_3	$C_{16}H_{11}N_3O_2S$	309	188-190	57
DNJ-1500-C	4-Cl	$C_{15}H_8ClN_3OS$	313	194-198	51
DNJ-1500-D	4-F	$C_{15}H_8FN_3OS$	297	190-192	56
DNJ-1500-E	4-NO_2	$C_{15}H_8N_4O_3S$	324	208-210	50

[f] Z. H. Chohan, H. Pervez, A. Rauf, K. M. Khan, C. T. Supuran; *J. Enz. Inhib. and Med. Chem.*, **2004**, *19 (5)*, 417.

3.4.6 GENERAL PROCEDURE FOR THE PREPARATION OF DNJ-1301 TO DNJ-1305

To a stirred solution of 0.01 mole of 2-propylpentanohydrazide in 50 ml warm ethanol was added 0.01 mole of an appropriate *N*-Mannich base of isatin in 60 ml ethanol. Then 2–3 drops of glacial acetic acid were added and the reaction mixture was refluxed for 5 hours. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using toluene : ethyl acetate (7 : 3) as a mobile phase. After the reaction to be completed, the flask was cooled to afford a solid product. The solid residue was filtered, washed with cold ethanol and dried. Recrystallization was carried out from hot ethanol. (Physical data of the synthesized end products are summarized in the table 3.5.1)

3.4.7 GENERAL PROCEDURE FOR THE PREPARATION OF DNJ-1401 TO DNJ-1405

It was again prepared by the method described by Chohan *et. al.* [f] To a stirred solution of 0.01 mole of 2-amino benzothiazole in 50 ml warm ethanol was added 0.01 mole an appropriate Mannich base of isatin in 60 ml ethanol. Then 2–3 drops of concentrated sulphuric acid were added and the reaction mixture was refluxed for 4 hours. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using hexane : ethyl acetate (6 : 4) as a mobile phase. After the reaction to be completed, the flask was cooled to afford a solid product. The solid residue was filtered, washed with cold ethanol, then with ether and dried. Recrystallization was carried out from hot ethanol. (Physical data of the synthesized end products are summarized in the table 3.5.2)

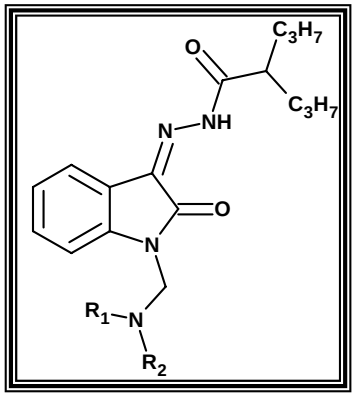
3.4.8 GENERAL PROCEDURE FOR THE PREPARATION OF DNJ-1501 TO DNJ-1505

It was prepared according to the method described by Jacobs *et. al.* [g] 0.01 mole of an appropriate Schiff base of isatin was charged into 50 ml round bottom flask. 3.5 ml acetic anhydride was added into it and the reaction mixture was refluxed for 4 hour with constant stirring. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using toluene : ethyl acetate (7 : 3) as a mobile phase. After the reaction to be completed, the flask was cooled to give desired product, which was washed with diethyl ether and dried. Recrystallization was carried out from methanol. (Physical data of the synthesized end products are summarized in the table 3.5.3)

[g] T. L. Jacobs, S. Winstein, G. B. Linden, J. H. Robson, E. F. Levy and D. Seymour; *Org. Synth.*, **1948**, *28*, 70.

3.5 PHYSICAL DATA TABLES

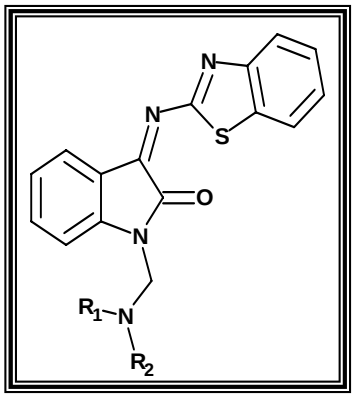
3.5.1 Physical data of *N*'-{1-[(substituted-1-yl) methyl]-2-oxo-1, 2-dihydro-3*H*-indol-3-ylidene}-2-propylpentanohydrazide (DNJ-1301 to DNJ-1305)



Code	Substitution R_1 & R_2	MF	MW (g/m)	MP (°C)	R_f	% Yield
DNJ-1301	Morpholine	$C_{21}H_{30}N_4O_3$	386	180-182	0.43	71
DNJ-1302	Piperidine	$C_{22}H_{32}N_4O_2$	384	176-178	0.41	69
DNJ-1303	1-Methyl Piperazine	$C_{22}H_{33}N_5O_2$	399	204-206	0.44	67
DNJ-1304	1-Ethyl Piperazine	$C_{23}H_{35}N_5O_2$	413	222-224	0.48	70
DNJ-1305	1-Benzyl Piperazine	$C_{28}H_{37}N_5O_2$	475	190-192	0.55	65

R_f value was calculated using solvent system = Toluene : Ethyl Acetate (7 : 3)

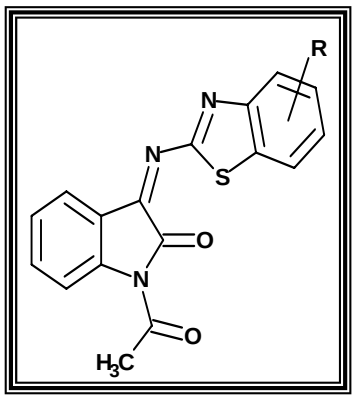
3.5.2 Physical data of 3-(1, 3-benzothiazol-2-ylimino)-1-(substituted-4-ylmethyl)-1, 3-dihydro-2*H*-indol-2-one (DNJ-1401 to DNJ-1405)



Code	Substitution R_1 & R_2	MF	MW (g/m)	MP (°C)	R_f	% Yield
DNJ-1401	Morpholine	$C_{20}H_{18}N_4O_2S$	378	230-232	0.40	60
DNJ-1402	Piperidine	$C_{21}H_{20}N_4OS$	376	210-212	0.43	58
DNJ-1403	1-Methyl Piperazine	$C_{21}H_{21}N_5OS$	391	196-198	0.45	63
DNJ-1404	1-Ethyl Piperazine	$C_{22}H_{23}N_5OS$	405	234-236	0.49	65
DNJ-1405	1-Benzyl Piperazine	$C_{27}H_{25}N_5OS$	467	200-202	0.56	57

R_f value was calculated using solvent system = Hexane : Ethyl Acetate (6 : 4)

3.5.3 Physical data of 1-acetyl-3-[((un)substituted-1, 3-benzothiazol-2-yl)imino]-1, 3-dihydro-2*H*-indol-2-one (DNJ-1501 to DNJ-1505)



Code	Substitution R	MF	MW (g/m)	MP (°C)	R_f	% Yield
DNJ-1501	4-H	$C_{17}H_{11}N_3O_2S$	321	178-180	0.36	57
DNJ-1502	4-OCH_3	$C_{18}H_{13}N_3O_3S$	351	172-174	0.39	65
DNJ-1503	4-Cl	$C_{17}H_{10}ClN_3O_2S$	355	216-218	0.37	63
DNJ-1504	4-F	$C_{17}H_{10}FN_3O_2S$	339	198-200	0.34	58
DNJ-1505	4-NO_2	$C_{17}H_{10}N_4O_4S$	366	204-206	0.38	60

R_f value was calculated using solvent system = Toluene : Ethyl Acetate (7 : 3)

3.6 SPECTRAL DISCUSSION

3.6.1 MASS SPECTRAL STUDY

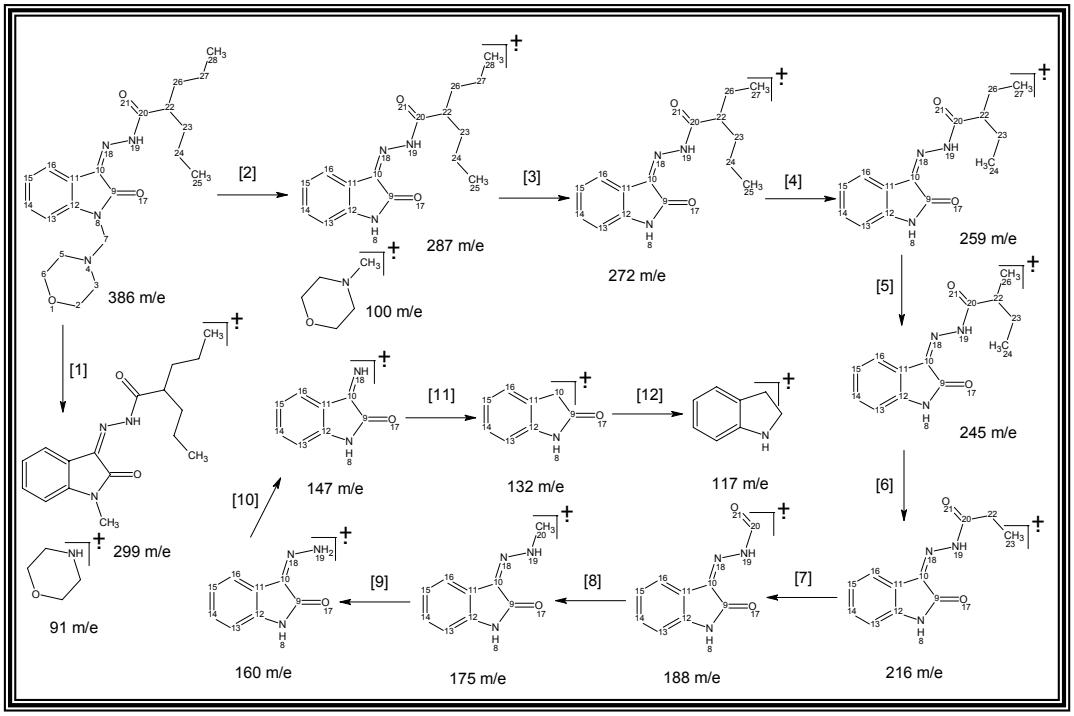
Mass spectra of the synthesized compounds were recorded on **Shimadzu GC-MS QP-2010** model using direct injection probe technique. The molecular ion peak was found in agreement with molecular weight of the respective compound. Characteristic M^{+2} ion peaks with one-third intensity of molecular ion peak were observed in case of compounds having chlorine atom. Fragmentation pattern can be observed to be particular for these compounds and the characteristic peaks obtained for each compound. Probable fragmentation pattern for DNJ-1301, DNJ-1401 and DNJ-1501 can be discussed as under.

_N_'-1-(morpholin-4-ylmethyl)-2-oxo-1, 2-dihydro-3_H_-indol-3-ylidene]-2-propylpentanohydrazide (DNJ-1301)

1. The target compound showed characteristic molecular ion peak.
2. C_7-N_4 bond cleavage gave characteristic peak at 299 m/e. [1]
3. C_7-N_8 bond cleavage gave two characteristic peaks at 287 m/e and 100 m/e (base peak). [2]
4. After C_7-N_8 bond cleavage, C_{27}-C_{28} bond cleavage and subsequently C_{24}-C_{25} bond cleavage gave characteristic peaks at 272 m/e and 259 m/e respectively. [3] & [4]
5. After C_{27}-C_{28} and C_{24}-C_{25} bond cleavages, C_{26}-C_{27} bond cleavage and subsequently C_{23}-C_{24} bond cleavage gave two characteristic peaks at 245 m/e and 216 m/e respectively. [5] & [6]
6. After C_{26}-C_{27} and C_{23}-C_{24} bond cleavages, C_{20}-C_{22} bond cleavage and subsequently C_{20}-C_{21} bond cleavage gave two characteristic peaks at 188 m/e and 175 m/e respectively. [7] & [8]
7. After C_{20}-C_{22} and C_{20}-C_{21} bond cleavages, N_{19}-C_{20} bond cleavage gave characteristic peak at 160 m/e. [9]

8. After N_{19}-C_{20} bond cleavage, N_{18}-N_{19} bond cleavage gave characteristic peak at 147 m/e. [10]
9. After N_{18}-N_{19} bond cleavage, C_{10}-N_{18} bond cleavage gave characteristic peak at 132 m/e. [11]
10. After C_{10}-N_{18} bond cleavage, C_9-O_{17} bond cleavage gave characteristic peak at 119 m/e. [12]

3.6.1.1 FRAGMENTATION PATTERN FOR DNJ-1301

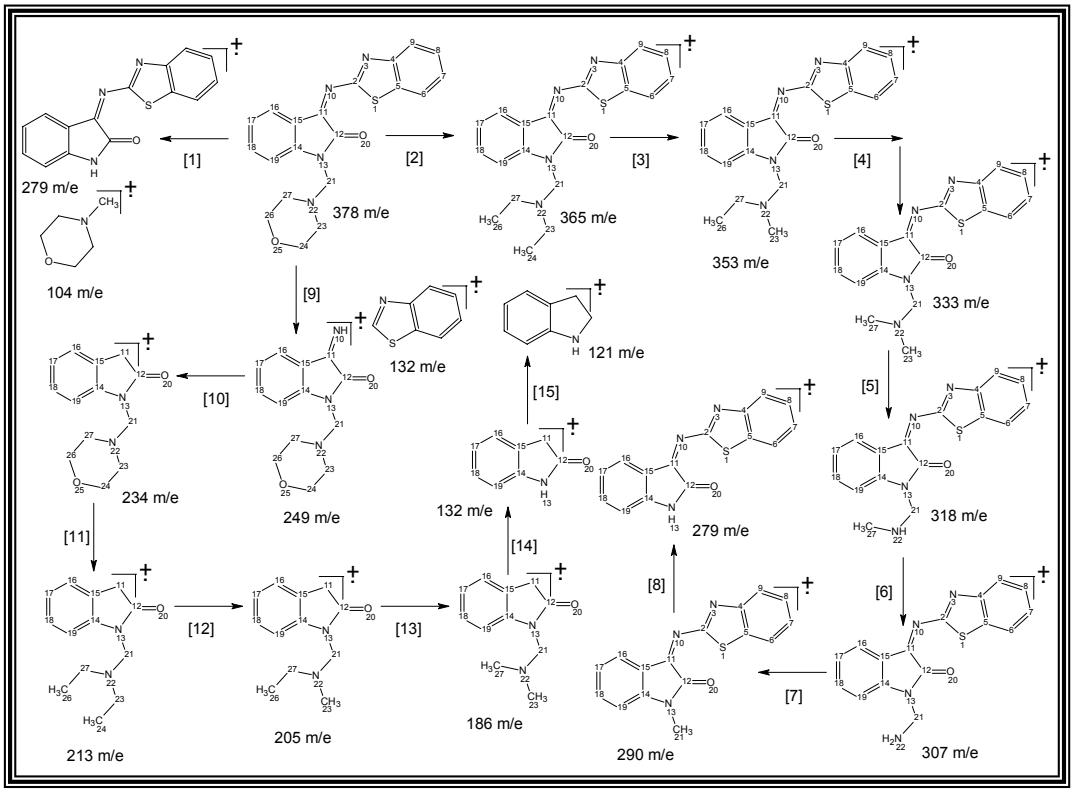


3-(1, 3-benzothiazol-2-ylimino)-1-(morpholin-4-ylmethyl)-1, 3-dihydro-2*H*-indol-2-one (DNJ-1401)

1. The target compound showed characteristic molecular ion peak.
2. C_{21}-N_{13} bond cleavage gave two characteristic peaks at 279 m/e and 104 m/e. [1]
3. O_{25}-C_{24} and O_{25}-C_{26} bond cleavages gave characteristic peak at 365 m/e. [2]

4. After O_{25}-C_{24} and O_{25}-C_{26} bond cleavages, C_{23}-C_{24} bond cleavage gave characteristic peak at 353 m/e. [3]
5. After C_{23}-C_{24} bond cleavage, C_{26}-C_{27} bond cleavage gave characteristic peak at 333 m/e. [4]
6. After C_{26}-C_{27} bond cleavage, N_{22}-C_{23} bond cleavage gave characteristic peak at 318 m/e. [5]
7. After N_{22}-C_{23} bond cleavage, N_{22}-C_{27} bond cleavage gave characteristic peak at 307 m/e. [6]
8. After N_{22}-C_{27} bond cleavage, C_{21}-N_{22} bond cleavage gave characteristic peak at 290 m/e (base peak). [7]
9. After C_{21}-N_{22} bond cleavage, N_{13}-C_{21} bond cleavage gave characteristic peak at 279 m/e. [8]
10. C_2-N_{10} bond cleavage gave two characteristic peaks at 249 m/e and 132 m/e. [9]
11. After C_2-N_{10} bond cleavage, N_{10}-C_{11} bond cleavage gave characteristic peak at 234 m/e. [10]
12. After N_{10}-C_{11} bond cleavage, O_{25}-C_{24} and O_{25}-C_{26} bond cleavages gave characteristic peak at 213 m/e. [11]
13. After O_{25}-C_{24} and O_{25}-C_{26} bond cleavages, C_{23}-C_{24} bond cleavage gave characteristic peak at 205 m/e. [12]
14. After C_{23}-C_{24} bond cleavage, C_{26}-C_{27} bond cleavage gave characteristic peak at 186 m/e. [13]
15. After C_{26}-C_{27} bond cleavage, N_{13}-C_{21} bond cleavage gave characteristic peak at 132 m/e. [14]
16. After N_{13}-C_{21} bond cleavage, C_{12}-O_{20} bond cleavage gave characteristic peak at 121 m/e. [15]

3.6.1.2 FRAGMENTATION PATTERN FOR DNJ-1401

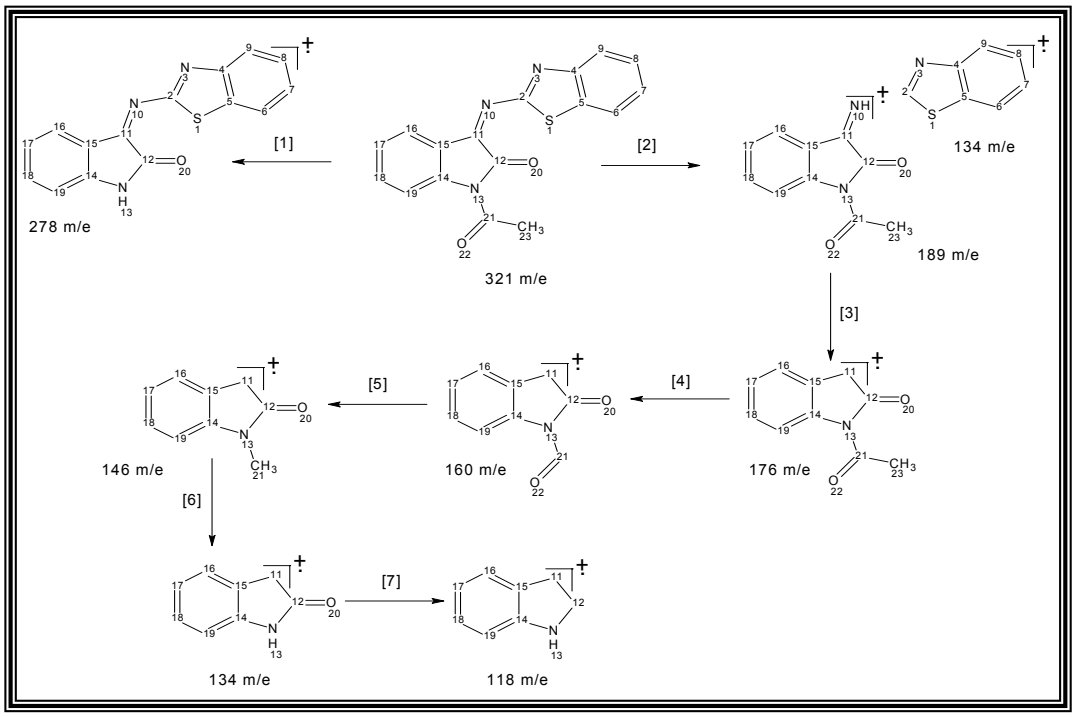


1-acetyl-3-(1, 3-benzothiazol-2-ylimino)-1, 3-dihydro-2*H*-indol-2-one (DNJ-1501)

1. The target compound showed characteristic molecular ion peak.
2. C_{21}-N_{13} bond cleavage gave characteristic peak at 278 m/e. [1]
3. C_2-N_{10} bond cleavage gave two characteristic peaks at 189 m/e (base peak) and 134 m/e. [2]
4. After C_2-N_{10} bond cleavage, C_{11}-N_{10} bond cleavage gave characteristic peak at 176 m/e. [3]
5. After C_{11}-N_{10} bond cleavage, C_{21}-C_{23} bond cleavage gave characteristic peak at 161 m/e. [4]
6. After C_{21}-C_{23} bond cleavage, C_{21}-C_{22} bond cleavage gave characteristic peak at 146 m/e. [5]

7. After C_{21}-C_{22} bond cleavage, N_{13}-C_{21} bond cleavage gave characteristic peak at 134 m/e. [6]
8. After N_{13}-C_{21} bond cleavage, C_{12}-O_{20} bond cleavage gave characteristic peak at 119 m/e. [7]

3.6.1.3 FRAGMENTATION PATTERN FOR DNJ-1401



3.6.2 IR SPECTRAL STUDY

IR spectra of the synthesized compounds were recorded on **Shimadzu FT IR 8400** spectrophotometer using Diffused Reflectance Attachment (DRA) System using Potassium Bromide.

N-H stretching frequency was observed near 3430 cm^{-1} in case of DNJ-1301 to DNJ-1305. There are two carbonyl groups present in all the compounds but due to the same environment, the peaks merged in the region of 1680 cm^{-1}. C=N stretching frequency was observed near 1610 cm^{-1} in all the compounds. C-N stretching (2° & 3°) frequency was found DNJ-1301 to

DNJ-1305 while rests of the compounds showed only C-N stretching (3°) frequency. C-H stretching frequencies were observed between 2810 cm^{-1} and 2970 cm^{-1}, while ring skeleton frequencies were observed near 1460 cm^{-1} in all the compounds.

Characteristic frequencies for p-di substitution were observed in DNJ-1502, DNJ-1503, DNJ-1504 and DNJ-1505. C-X (X = Cl, F) stretching frequencies were obtained in DNJ-1503 and DNJ-1504 while DNJ-1502 showed characteristic C-O-C stretching frequency. DNJ-1301 and DNJ-1401 also showed C-O-C stretching frequency.

3.6.3 ^{1}H & ^{13}C NMR SPECTRAL STUDY

^{1}H & ^{13}C NMR spectra of the synthesized compounds were recorded on **Bruker Avance II 400** spectrometer. Sample solutions were made in $CDCl_3$ solvent using tetramethylsilane (TMS) as the internal standard unless otherwise mentioned. Numbers of protons and numbers of carbons identified from proton NMR & carbon NMR spectrum and their chemical shift (δ ppm) were in the agreement of the structure of the molecule. *J* values were calculated to identify o, m and p coupling. In some cases, aromatic protons were obtained as multiplet. ^{1}H & ^{13}C NMR spectral interpretation can be discussed as under.

^{1}H NMR spectral interpretation of *N*'-[1-(morpholin-4-ylmethyl)-2-oxo-1, 2-dihydro-3*H*-indol-3-ylidene]-2-propylpentanohydrazide (DNJ-1301)

1. Four protons of C_{23} and C_{26} in the hydrazide linkage gave multiplet at 1.79 δ ppm. While another four protons of C_{24} and C_{27}gave quartet at 1.53 δ ppm. Six protons of two methyl groups C_{25} and C_{28} should showed triplet at 1.37 δ ppm.
2. Another four protons of morpholinyl methylene groups (C_3 & C_5) and one proton of C_{22} gave multiplet at 2.90 δ ppm. Due to the nitrogen

atmosphere, four protons of methylene groups flipped individually and peaks got merged.

3. Four protons of two morpholinyl methylene groups (C_2 & C_6) gave quintet at 3.69 δ ppm.
4. Two protons of methylene group (C_7) attached to two nitrogen atoms of indolinone and morpholine ring gave singlet at 4.48 δ ppm.
5. There are four aromatic protons in the molecule. One proton of C_{13} gave quintet (triplet-doublet) at 7.15 δ ppm. One proton of C_{15} gave quartet at 7.39 δ ppm while one proton of C_{14} gave doublet at 7.62 δ ppm. Rest of the proton of C_{16} gave doublet at 7.80 δ ppm.
6. One most deshielded proton of secondary amine in hydrazide linkage (-NH) gave singlet in the down field at 12.52 δ ppm.

^{13}C NMR spectral interpretation of *N*'-[1-(morpholin-4-ylmethyl)-2-oxo-1, 2-dihydro-3H-indol-3-ylidene]-2-propylpentanohydrazide (DNJ-1301)

1. Two most shielded methyl carbons of n-propyl chain (C_{25} & C_{28}) showed peak at 14.07 δ ppm.
2. Two methylene carbons of n-propyl chain (C_{24} & C_{27}) showed peak at 20.68 δ ppm.
3. Another two methylene carbons (C_{23} & C_{26}) showed peak at 34.58 δ ppm.
4. Methine carbon of hydrazide linkage (C_{22}) showed peak at 51.00 δ ppm.
5. Two methylene carbons (C_3 & C_5) of morpholinyl ring gave peak at 51.94 δ ppm. While another two methylene carbons of morpholinyl ring (C_2 & C_6) showed peak at 66.92 δ ppm.
6. Methylene carbon (C_7) attached to both the nitrogen of indole nucleus and morpholine nucleus gave peak at 66.59 δ ppm.
7. Peaks obtained at 76.89, 77.21, 77.53 δ ppm are due to the solvent $CDCl_3$.
8. Peaks obtained at 110.44, 119.53, 119.95, 120.33, 136.04 and 143.19 δ ppm are due to the aromatic carbons C_{13}, C_{15}, C_{16}, C_{11}, C_{14} and C_{12} respectively.

9. Peak obtained at 161.61 δ ppm is due to the azomethine carbon C_{10}.
10. Carbonyl carbon (C_9) of indolinone ring became deshielded due to the adjacent nitrogen atom and showed peak in down field at 173.69 δ ppm.
11. Carbonyl carbon of amide group in hydrazide linkage (C_{20}) became most deshielded and showed peak in down field at 179.30 δ ppm.

^{1}H NMR spectral interpretation of 3-(1, 3-benzothiazol-2-ylimino)-1-(morpholin-4-ylmethyl)-1, 3-dihydro-2*H*-indol-2-one (DNJ-1401)

1. Four protons of two methylene groups (C_{23} & C_{27}) of morpholine ring gave triplet at 2.36 δ ppm.
2. Another four protons of two methylene groups (C_{24} & C_{26}) of morpholine ring gave triplet at 2.60 δ ppm.
3. Two protons of methylene group (C_{21}) gave singlet at 3.76 δ ppm.
4. Rests of the peaks are due to the aromatic protons which are obtained as multiplet and are in the agreement of the structure of the molecule. Two protons of C_7 and C_{19} gave quintet at 6.97 δ ppm. Two protons of C_8 and C_{18} gave triplet at 7.11 δ ppm, while one proton of C_{16} gave multiplet at 7.33 δ ppm. Rests of the three protons of C_6, C_9 and C_{17} gave multiplet at 7.43 δ ppm.

^{13}C NMR spectral interpretation of 3-(1, 3-benzothiazol-2-ylimino)-1-(morpholin-4-ylmethyl)-1, 3-dihydro-2*H*-indol-2-one (DNJ-1401)

1. Two methylene carbons (C_{23} & C_{27}) of morpholinyl ring gave peak at 46.55 δ ppm. While another two methylene carbons of morpholinyl ring (C_{24} & C_{26}) showed peak at 66.89 δ ppm.
2. Methylene carbon (C_{21}) attached to both the nitrogen of indole nucleus and morpholine nucleus gave peak at 66.66 δ ppm.
3. Peaks obtained at 76.89, 77.21, 77.53 δ ppm are due to the solvent $CDCl_3$.

4. Peaks obtained at 110.50, 119.55, 119.94, 120.30, 121.71, 123.28, 123.61, 132.01, 136.04, 143.22 and 162.32 δ ppm are due to the aromatic carbons C_{19}, C_{16}, C_{15}, C_9, C_{17}, C_6, C_7, C_8, C_5, C_{18}, C_{14} and C_4 respectively.
5. Azomethine carbon (C_{11}) gave peak at 161.61 δ ppm.
6. Carbonyl carbon (C_{12}) of indolinone ring became deshielded due to the adjacent nitrogen atom and showed peak in the down field at 173.69 δ ppm.
7. C_2 carbon of thiazole ring became most deshielded due to the neighboring two nitrogen atoms and showed peak in the down field at 179.32 δ ppm.

^{1}H NMR spectral interpretation of 1-acetyl-3-(1, 3-benzothiazol-2-ylimino)-1, 3-dihydro-2*H*-indol-2-one (DNJ-1501)

1. Three shielded protons of methyl group (C_{23}) gave singlet in the up field at 3.77 δ ppm.
2. Rests of the peaks are due to the aromatic protons which are obtained as multiplet and are in the agreement of the structure of the molecule. Two protons of C_7 and C_{17} gave quintet at 6.97 δ ppm. Two protons of C_8 and C_{18} gave triplet at 7.11 δ ppm, while another two protons of C_6 and C_9 gave multiplet at 7.33 δ ppm. Rests of the two protons of C_{16} and C_{19} gave multiplet at 7.43 δ ppm.

^{13}C NMR spectral interpretation of 1-acetyl-3-(1, 3-benzothiazol-2-ylimino)-1, 3-dihydro-2*H*-indol-2-one (DNJ-1501)

1. Methyl group (C_{23}) attached to the carbonyl group (C_{21}) became most shielded and showed peak at 20.68 δ ppm in the up field.
2. Peaks obtained at 76.91, 77.23, 77.51 δ ppm are due to the solvent $CDCl_3$.
3. Peaks obtained at 119.95, 120.33, 121.71, 123.28, 123.61, 130.80, 131.36, 132.01, 136.08, 140.04, 146.08 and 146.58 δ ppm are due to

the aromatic carbons C_9, C_6, C_{16}, C_{19}, C_7, C_8, C_{17}, C_{15}, C_{18}, C_5, C_{14} and C_4 respectively.

4. Azomethine carbon (C_{11}) gave peak at 152.69 δ ppm.
5. Two carbonyl carbons (C_{12} & C_{21}) of indolinone ring became deshielded due to the neighboring nitrogen atom and showed peak in the down field at 161.59 and 162.30 δ ppm respectively.
6. C_2 carbon of thiazole ring became most deshielded due to the neighboring two nitrogen atoms and showed peak in the down field at 179.34 δ ppm.

3.6.4 ELEMENTAL ANALYSIS

Elemental analysis of the synthesized compounds was carried out on **Vario EL Carlo Erba 1108** which showed calculated and found percentage values of Carbon, Hydrogen and Nitrogen in support of the structure of synthesized compounds. The spectral and elemental analysis data are given for individual compounds.

3.7 ANALYTICAL DATA

***N*'-[1-(MORPHOLIN-4-YL METHYL)-2-OXO-1, 2-DIHYDRO-3*H*-INDOL-3-YLIDENE]-2-PROPYLPENTANOHYDRAZIDE (DNJ-1301):** IR (KBr, cm^{-1}): 3432 (-NH), 2955 ($-CH_3$), 2862 ($-CH_2$), 1680 (>CO), 1610 (-C=N), 1458 (Ar-H, ben), 1311 (C-N, Ar, 2°), 1213 (C-N, Aliphatic, 3°), 1050 (C-O-C); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.37 (6H, t), 1.53 (4H, qt), 1.79 (4H, m), 2.90 (4H, m), 3.69 (4H, q), 4.48 (2H, s), 7.15 (1H, q, *J* = 7.92 Hz), 7.39 (1H, qt), 7.62 (1H, d, *J* = 7.28 Hz), 7.80 (1H, d, *J* = 7.36 Hz), 12.52 (1H, s); ^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm) 179.30, 173.69, 161.61, 143.19, 136.04, 120.33, 119.95, 119.53, 110.44, 66.92, 66.59, 51.94, 51.00, 34.58, 20.68, 14.07; MS m/z = 386 (M^+); Anal. Calcd. for $C_{21}H_{30}N_4O_3$: C, 65.26; H, 7.82; N, 14.50. Found: C, 65.21; H, 7.85; N, 14.47.

***N*'-[2-OXO-1-(PIPERIDIN-1-YL METHYL)-1, 2-DIHYDRO-3*H*-INDOL-3-YLIDENE]-2-PROPYLPENTANOHYDRAZIDE (DNJ-1302):** IR (KBr, cm^{-1}): 3430 (-NH), 2957 ($-CH_3$), 2859 ($-CH_2$), 1682 (>CO), 1608 (-C=N), 1460 (Ar-H, ben), 1309 (C-N, Ar, 2°), 1210 (C-N, Aliphatic, 3°), 1055 (C-O-C); MS m/z = 384 (M^+); Anal. Calcd. for $C_{22}H_{32}N_4O_2$: C, 68.72; H, 8.39; N, 14.57. Found: C, 68.76; H, 8.43; N, 14.53.

***N*'-{1-[(4-METHYL PIPERAZIN-1-YL) METHYL]-2-OXO-1, 2-DIHYDRO-3*H*-INDOL-3-YLIDENE}-2-PROPYLPENTANOHYDRAZIDE (DNJ-1303):** IR (KBr, cm^{-1}): 3428 (-NH), 2962 ($-CH_3$), 2854 ($-CH_2$), 1679 (>CO), 1605 (-C=N), 1464 (Ar-H, ben), 1311 (C-N, Ar, 2°), 1214 (C-N, Aliphatic, 3°), 1053 (C-O-C); MS m/z = 399 (M^+); Anal. Calcd. for $C_{22}H_{33}N_5O_2$: C, 66.14; H, 8.33; N, 17.53. Found: C, 66.10; H, 8.37; N, 17.50.

***N*'-{1-[(4-ETHYL PIPERAZIN-1-YL) METHYL]-2-OXO-1, 2-DIHYDRO-3*H*-INDOL-3-YLIDENE}-2-PROPYLPENTANOHYDRAZIDE (DNJ-1304):** IR (KBr, cm^{-1}): 3431 (-NH), 2960 ($-CH_3$), 2856 ($-CH_2$), 1682 (>CO), 1607 (-C=N), 1462 (Ar-H, ben), 1310 (C-N, Ar, 2°), 1212 (C-N, Aliphatic, 3°), 1051 (C-O-C);

MS m/z = 413 (M^+); Anal. Calcd. for $C_{23}H_{35}N_5O_2$: C, 66.80; H, 8.53; N, 16.93. Found: C, 66.85; H, 8.57; N, 16.89.

***N*'-{1-[(4-BENZYL PIPERAZIN-1-YL) METHYL]-2-OXO-1, 2-DIHYDRO-3*H*-INDOL-3-YLIDENE}-2-PROPYLPENTANOHYDRAZIDE (DNJ-1305):** IR (KBr, cm^{-1}): 3429 (-NH), 2965 ($-CH_3$), 2861 ($-CH_2$), 1683 (>CO), 1610 (-C=N), 1463 (Ar-H, ben), 1313 (C-N, Ar, 2°), 1210 (C-N, Aliphatic, 3°), 1050 (C-O-C); MS m/z = 475 (M^+); Anal. Calcd. for $C_{28}H_{37}N_5O_2$: C, 70.71; H, 7.84; N, 14.72. Found: C, 70.66; H, 7.88; N, 14.76.

3-[(6-METHOXY-1, 3-BENZOTHIAZOL-2-YL) IMINO]-1-(MORPHOLIN-4-YL METHYL)-1, 3-DIHYDRO-2*H*-INDOL-2-ONE (DNJ-1401): IR (KBr, cm^{-1}): 2956 ($-CH_3$), 2859 ($-CH_2$), 1683 (>CO), 1613 (-C=N), 1464 (Ar-H, ben), 1344 (C-N, Ar, 3°), 1250, (C-S-C), 1212 (C-N, Aliphatic, 3°), 1049 (C-O-C); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 2.36 (4H, t), 2.60 (4H, t), 3.76 (2H, s), 6.97 (2H, q, *J* = 0.6 Hz, *J* = 1.16 Hz), 7.11 (2H, t), 7.33 (1H, m, *J* = 1.68 Hz, *J* = 1.08 Hz, *J* = 1.92 Hz, *J* = 0.52 Hz), 7.43 (3H, m, *J* = 1.48 Hz, *J* = 2.24 Hz); ^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm) 179.32, 173.69, 162.32, 161.61, 143.22, 136.04, 132.01, 123.61, 123.28, 121.71, 120.30, 119.94, 119.55, 110.50, 66.89, 66.66, 46.55; MS m/z = 408 (M^+); Anal. Calcd. for $C_{21}H_{20}N_4O_3S$: C, 61.75; H, 4.94; N, 13.72. Found: C, 61.70; H, 4.90; N, 13.68.

3-[(6-METHOXY-1, 3-BENZOTHIAZOL-2-YL) IMINO]-1-(PIPERIDIN-1-YL METHYL)-1, 3-DIHYDRO-2*H*-INDOL-2-ONE (DNJ-1402): IR (KBr, cm^{-1}): 2958 ($-CH_3$), 2860 ($-CH_2$), 1680 (>CO), 1610 (-C=N), 1466 (Ar-H, ben), 1340 (C-N, Ar, 3°), 1248, (C-S-C), 1210 (C-N, Aliphatic, 3°); MS m/z = 406 (M^+); Anal. Calcd. for $C_{22}H_{22}N_4O_2S$: C, 65.00; H, 5.46; N, 13.78. Found: C, 64.95; H, 5.50; N, 13.82.

3-[(6-METHOXY-1, 3-BENZOTHIAZOL-2-YL) IMINO]-1-[(4-METHYL PIPERAZIN-1-YL) METHYL]-1, 3-DIHYDRO-2*H*-INDOL-2-ONE (DNJ-1403): IR (KBr, cm^{-1}): 2962 ($-CH_3$), 2856 ($-CH_2$), 1681 (>CO), 1615 (-C=N), 1467 (Ar-H, ben), 1341 (C-N, Ar, 3°), 1252, (C-S-C), 1214 (C-N, Aliphatic, 3°); MS m/z

= 421 (M^+); Anal. Calcd. for $C_{22}H_{23}N_5O_2S$: C, 62.69; H, 5.50; N, 16.61. Found: C, 62.73; H, 5.44; N, 16.65.

1-[(4-ETHYL PIPERAZIN-1-YL) METHYL]-3-[(6-METHOXY-1, 3-BENZOTHIAZOL-2-YL) IMINO]-1, 3-DIHYDRO-2*H*-INDOL-2-ONE (DNJ-1404): IR (KBr, cm^{-1}): 2959 (-CH_3), 2854 (-CH_2), 1685 (>CO), 1610 (-C=N), 1466 (Ar-H, ben), 1342 (C-N, Ar, 3°), 1253, (C-S-C), 1214 (C-N, Aliphatic, 3°); MS m/z = 435 (M^+); Anal. Calcd. for $C_{23}H_{25}N_5O_2S$: C, 63.43; H, 5.79; N, 16.08. Found: C, 63.40; H, 5.74; N, 16.03.

1-[(4-BENZYL PIPERAZIN-1-YL) METHYL]-3-[(6-METHOXY-1, 3-BENZOTHIAZOL-2-YL) IMINO]-1, 3-DIHYDRO-2*H*-INDOL-2-ONE (DNJ-1405): IR (KBr, cm^{-1}): 2961 (-CH_3), 2856 (-CH_2), 1683 (>CO), 1610 (-C=N), 1466 (Ar-H, ben), 1344 (C-N, Ar, 3°), 1251, (C-S-C), 1214 (C-N, Aliphatic, 3°); MS m/z = 497 (M^+); Anal. Calcd. for $C_{28}H_{27}N_5O_2S$: C, 67.58; H, 5.47; N, 14.07. Found: C, 67.54; H, 5.44; N, 14.10.

1-ACETYL-3-(1, 3-BENZOTHIAZOL-2-YL IMINO)-1, 3-DIHYDRO-2*H*-INDOL-2-ONE (DNJ-1501): IR (KBr, cm^{-1}): 2956 (-CH_3), 2858 (-CH_2), 1683 (>CO), 1609 (-C=N), 1464 (Ar-H, ben), 1342 (C-N, Ar, 3°), 1252, (C-S-C), 1213 (C-N, Aliphatic, 3°); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 3.77 (3H, s), 6.97 (2H, m, *J* = 1.12 Hz, *J* = 0.80 Hz), 7.11 (2H, t), 7.33 (2H, m, *J* = 0.44 Hz, *J* = 1.28 Hz), 7.43 (2H, m, *J* = 1.52 Hz, *J* = 1.44 Hz); ^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm) 179.34, 162.30, 161.59, 152.69, 146.58, 146.08, 140.04, 136.08, 132.01, 131.36, 130.80, 123.61, 123.28, 121.71, 120.33, 119.95, 20.68; MS m/z = 321 (M^+); Anal. Calcd. for $C_{17}H_{11}N_3O_2S$: C, 63.54; H, 3.45; N, 13.08. Found: C, 63.50; H, 3.41; N, 13.02.

1-ACETYL-3-[(6-METHOXY-1, 3-BENZOTHIAZOL-2-YL) IMINO]-1, 3-DIHYDRO-2*H*-INDOL-2-ONE (DNJ-1502): IR (KBr, cm^{-1}): 2962 (-CH_3), 2859 (-CH_2), 1681 (>CO), 1613 (-C=N), 1471 (Ar-H, ben), 1341 (C-N, Ar, 3°), 1252, (C-S-C), 1217 (C-N, Aliphatic, 3°), 1055 (C-O-C); MS m/z = 351 (M^+); Anal. Calcd. for $C_{18}H_{13}N_3O_3S$: C, 61.53; H, 3.73; N, 11.96. Found: C, 61.57; H, 3.77; N, 12.00.

1-ACETYL-3-[(6-CHLORO-1, 3-BENZOTHIAZOL-2-YL) IMINO]-1, 3-DIHYDRO-2*H*-INDOL-2-ONE (DNJ-1503): IR (KBr, cm^{-1}): 2960 ($-CH_3$), 2856 ($-CH_2$), 1691 (>CO), 1612 (-C=N), 1467 (Ar-H, ben), 1339 (C-N, Ar, 3°), 1246, (C-S-C), 1209 (C-N, Aliphatic, 3°), 870 (C-Cl), 815 (1,4-di sub); MS m/z = 355 (M^+); Anal. Calcd. for $C_{17}H_{10}ClN_3O_2S$: C, 57.39; H, 2.83; N, 11.81. Found: C, 57.43; H, 2.86; N, 11.86.

1-ACETYL-3-[(6-FLUORO-1, 3-BENZOTHIAZOL-2-YL) IMINO]-1, 3-DIHYDRO-2*H*-INDOL-2-ONE (DNJ-1504): IR (KBr, cm^{-1}): 2958 ($-CH_3$), 2860 ($-CH_2$), 1689 (>CO), 1610 (-C=N), 1470 (Ar-H, ben), 1340 (C-N, Ar, 3°), 1250, (C-S-C), 1215 (C-N, Aliphatic, 3°), 992 (C-F), 810 (1,4-di sub); MS m/z = 339 (M^+); Anal. Calcd. for $C_{17}H_{10}FN_3O_2S$: C, 60.17; H, 2.97; N, 12.38. Found: C, 60.13; H, 2.93; N, 12.33.

1-ACETYL-3-[(6-NITRO-1, 3-BENZOTHIAZOL-2-YL) IMINO]-1, 3-DIHYDRO-2*H*-INDOL-2-ONE (DNJ-1505): IR (KBr, cm^{-1}): 2955 ($-CH_3$), 2850 ($-CH_2$), 1685 (>CO), 1613 (-C=N), 1463 (Ar-H, ben), 1339 (C-N, Ar, 3°), 1248, (C-S-C), 1211 (C-N, Aliphatic, 3°), 812 (1,4-di sub); MS m/z = 366 (M^+); Anal. Calcd. for $C_{17}H_{10}N_4O_4S$: C, 55.73; H, 2.75; N, 15.29. Found: C, 55.77; H, 2.80; N, 15.33.

3.8 RESULTS AND DISCUSSION

Mannich bases and Schiff bases of isatin were prepared in this chapter. Isatin is known to produce *N*-Mannich bases using formaldehyde and secondary amines while it is known to give 3-substiuted Schiff bases when reacted with primary amine.

Isatin-*N*-Mannich bases were prepared using the known methodology and their Schiff bases were prepared using two different primary amine functional groups, one is 2-propylpentanohydrazide and another is 2-aminobenzothiazole. 2-propylpentanohydrazide was prepared by reacting hydrazine hydrate with Methyl-2-propylpentanoate and 2-propylpentanoate was prepared by the esterification of 2-propylpentanoic acid which is also known as very well known anticonvulsant drug “Valproic acid”. 2-aminobenzothiazoles also possess varied biological profile. Thus two biologically active components were substituted on isatin to study the biological activity profile of title compounds.

Schiff base of isatin using 2-propylpenanohydrazide is not reported so far while Schiff base of isatin and 2-aminobenzothiazole is not much explored. Moreover Mannich bases of these Schiff bases are not reported so far. Furthermore acetylation was carried out on isatin-Schiff bases. Thus it is first attempt to prepare three different types of indolinone derivatives and their anticancer evaluation.

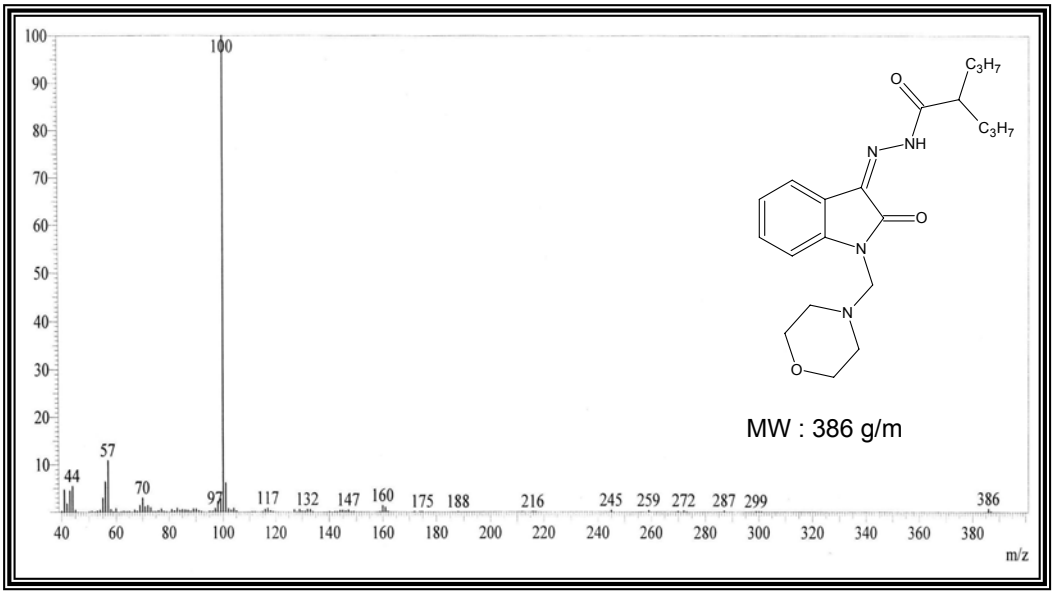
The compounds prepared in this chapter possess indole nucleus and are basically indolinone derivatives. Indoles and indolinones are very well known for their anticancer activity. Thus all the compounds were screened for their anticancer activity against three different cancer cell lines. (*viz.* breast, lung and stomach) All the compounds found to be good to moderate active against all the three cancer cell lines.

3.9 CONCLUSION

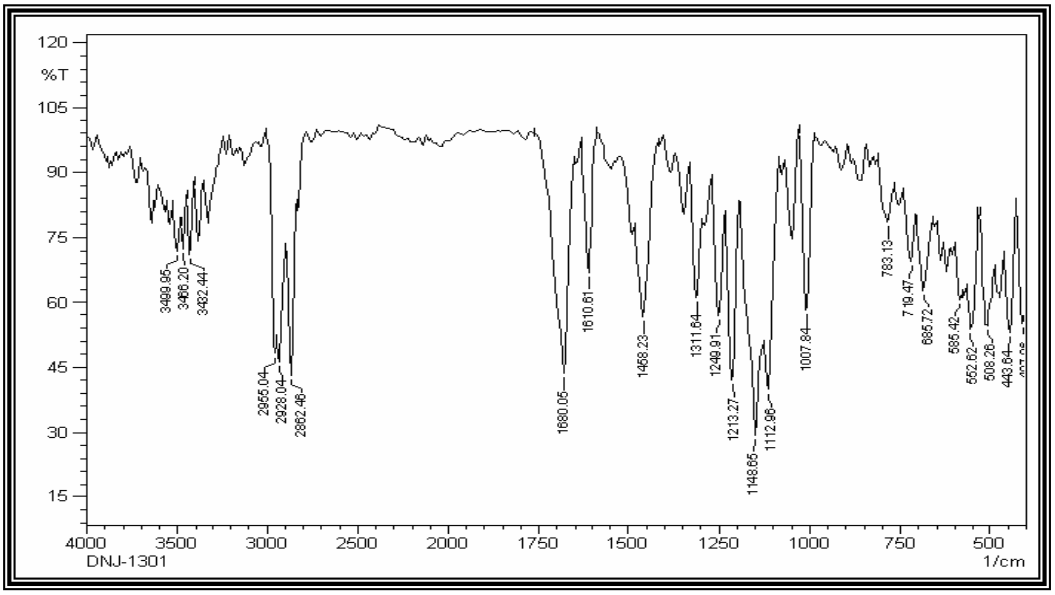
Indole nucleus is much known for its anticancer activity. Many naturally occurring indole alkaloids as well as synthetic indole molecules have shown pronounced anticancer activity as well as other biological profile too. Exploration of unreported chemistry and their biological activity was the aim behind the work done in this chapter.

3.10 SPECTRAL REPRESENTATION OF SYNTHESIZED COMPOUNDS

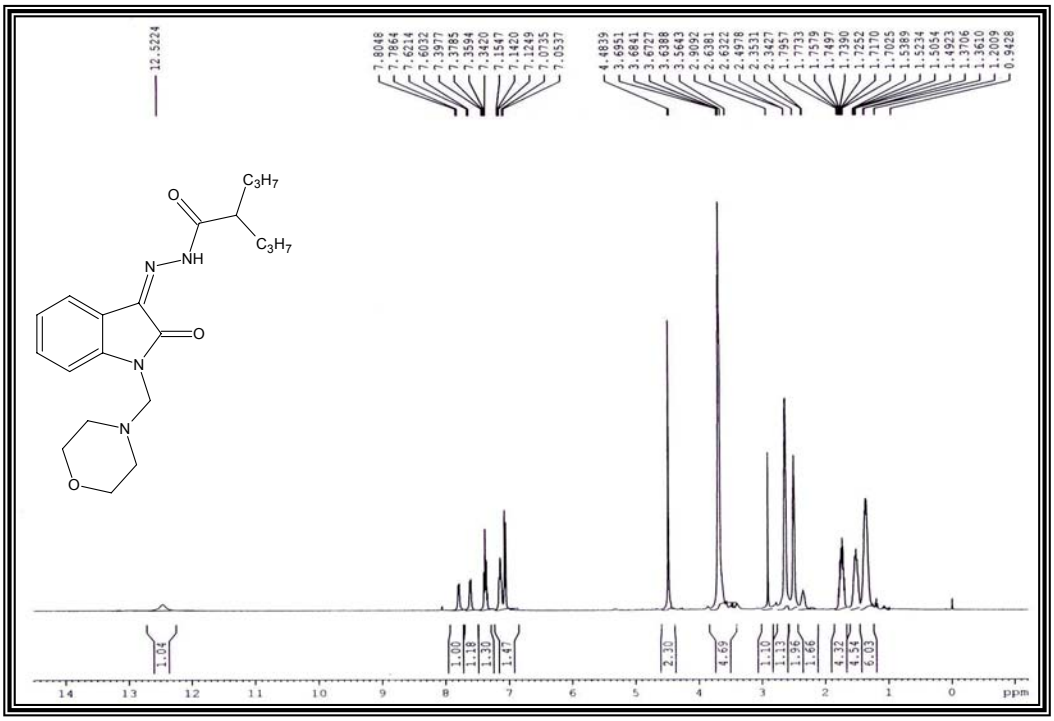
3.10.1 Mass Spectrum of DNJ-1301

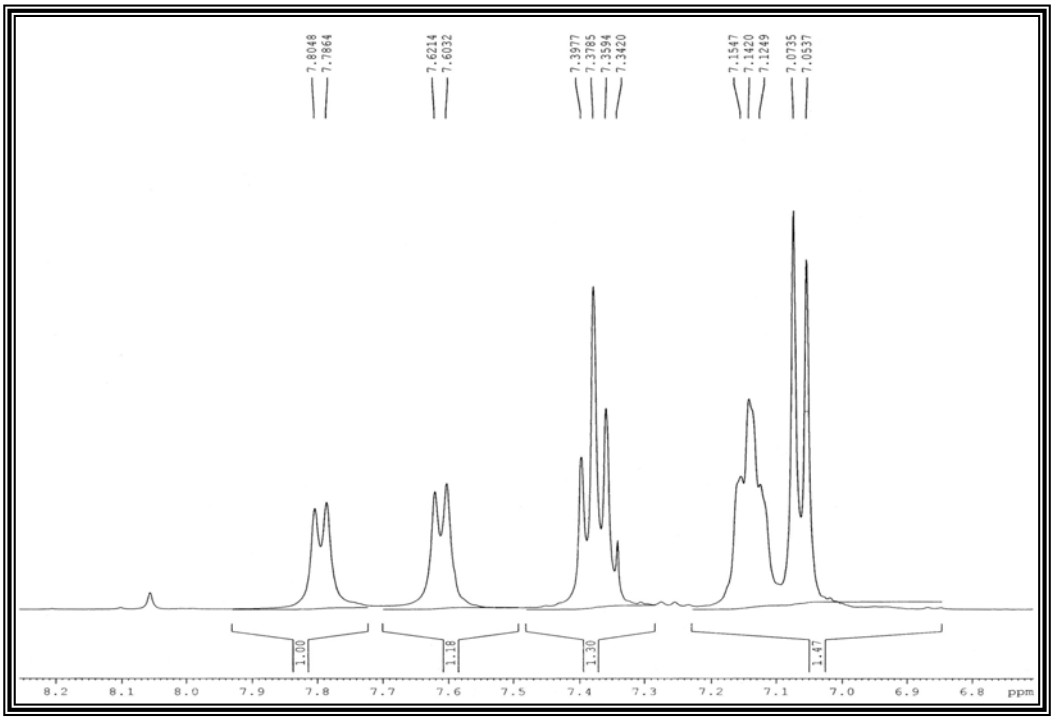


3.10.2 IR Spectrum of DNJ-1301

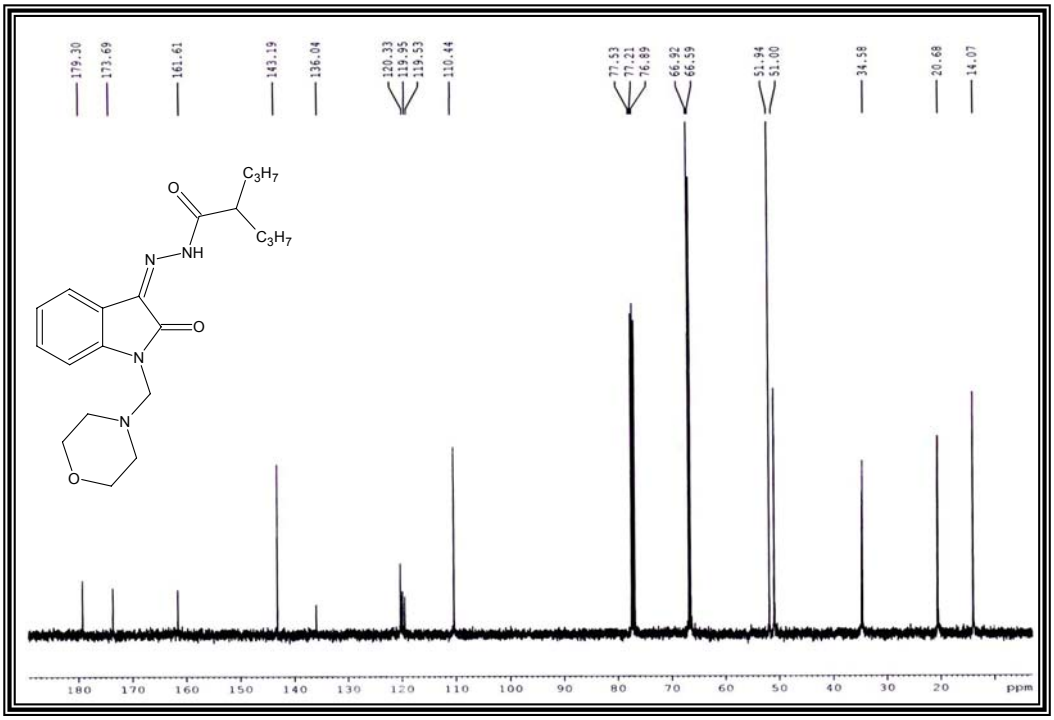


3.10.3 ^{1}H NMR Spectrums of DNJ-1301

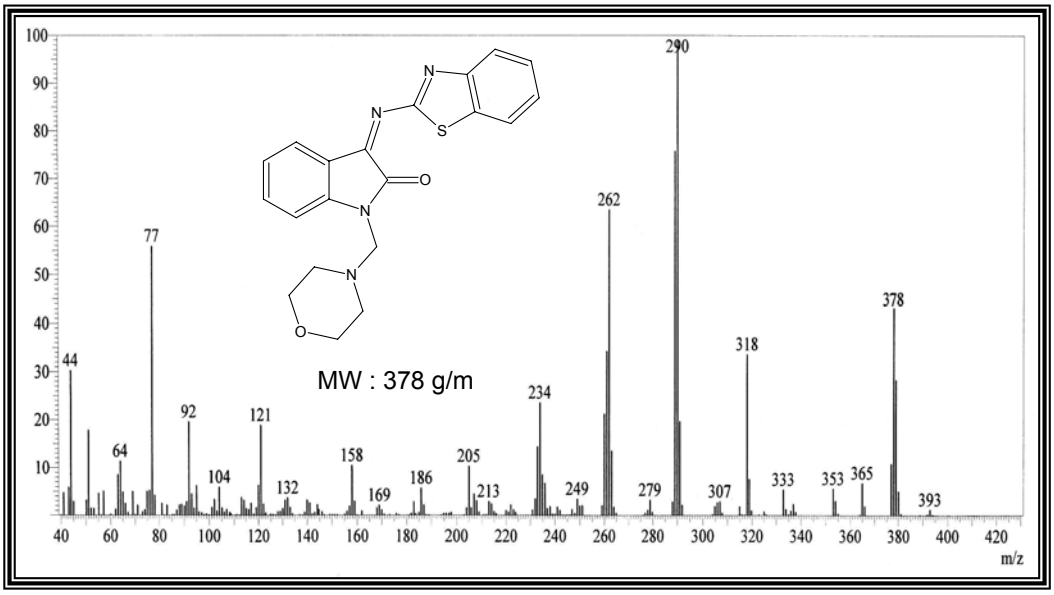




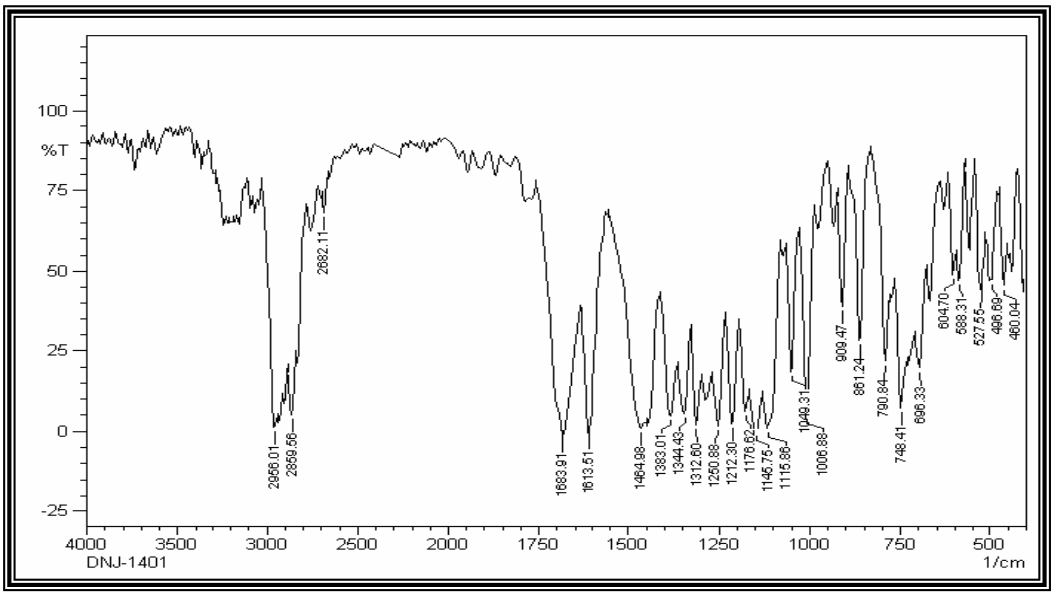
3.10.4 ^{13}C NMR Spectrum of DNJ-1301



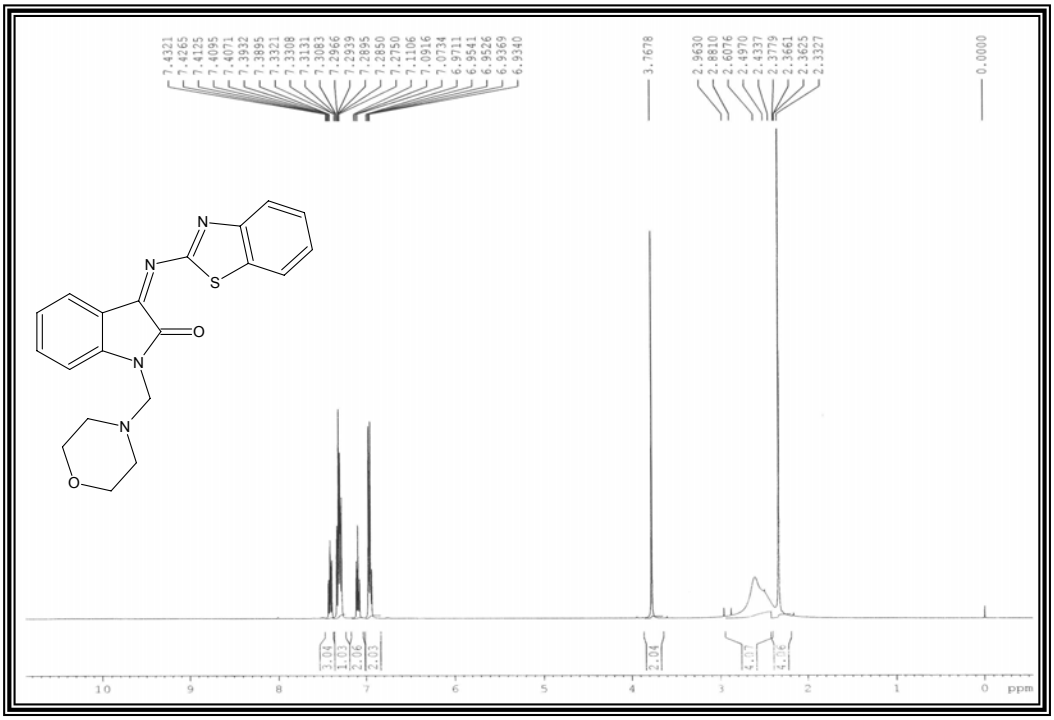
3.10.5 Mass Spectrum of DNJ-1401

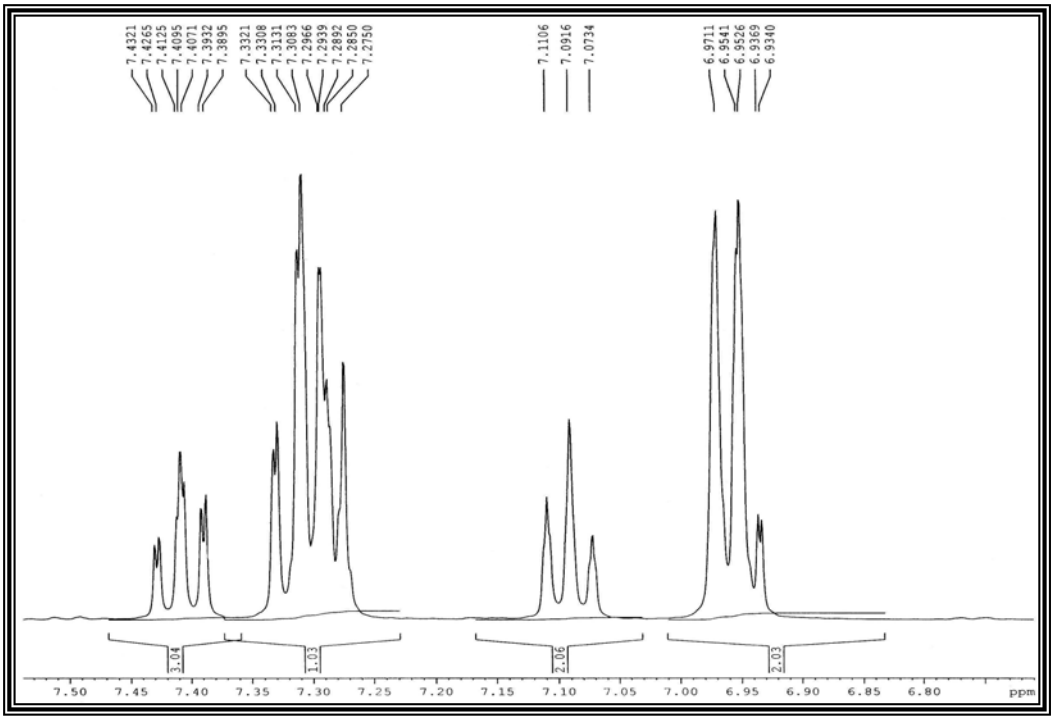


3.10.6 IR Spectrum of DNJ-1401

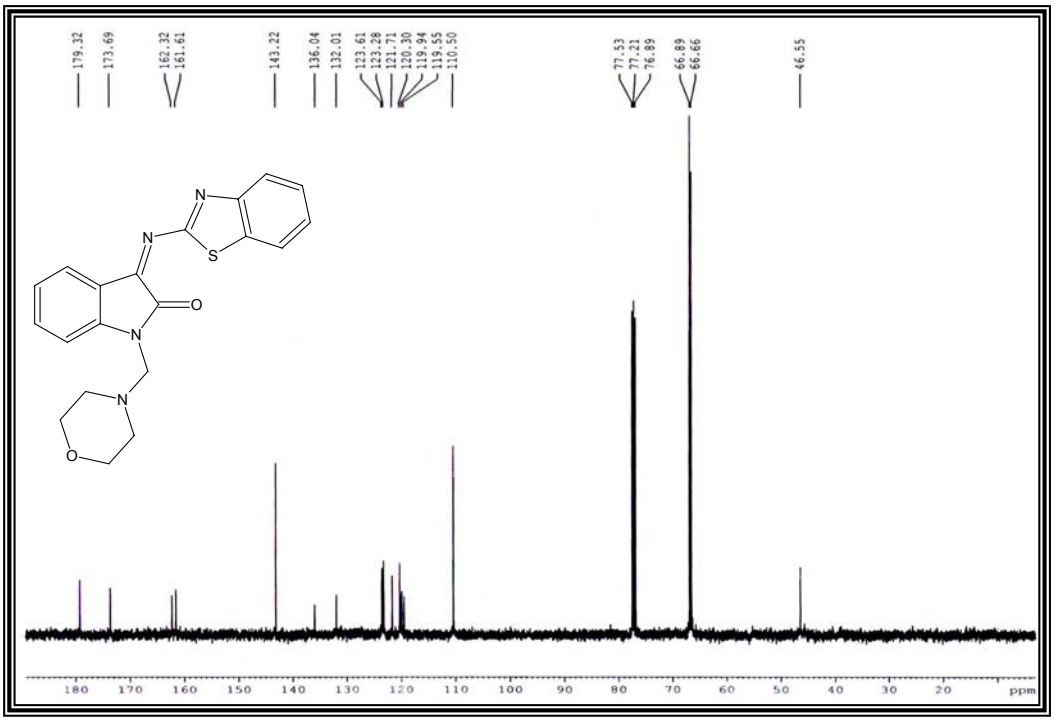


3.10.7 ^{1}H NMR Spectrum of DNJ-1401

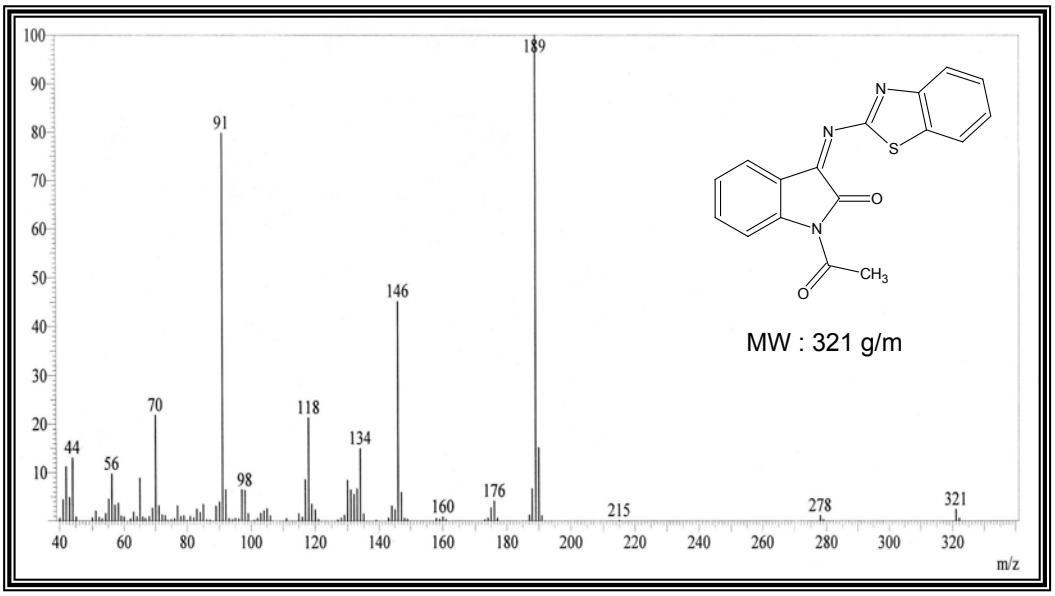




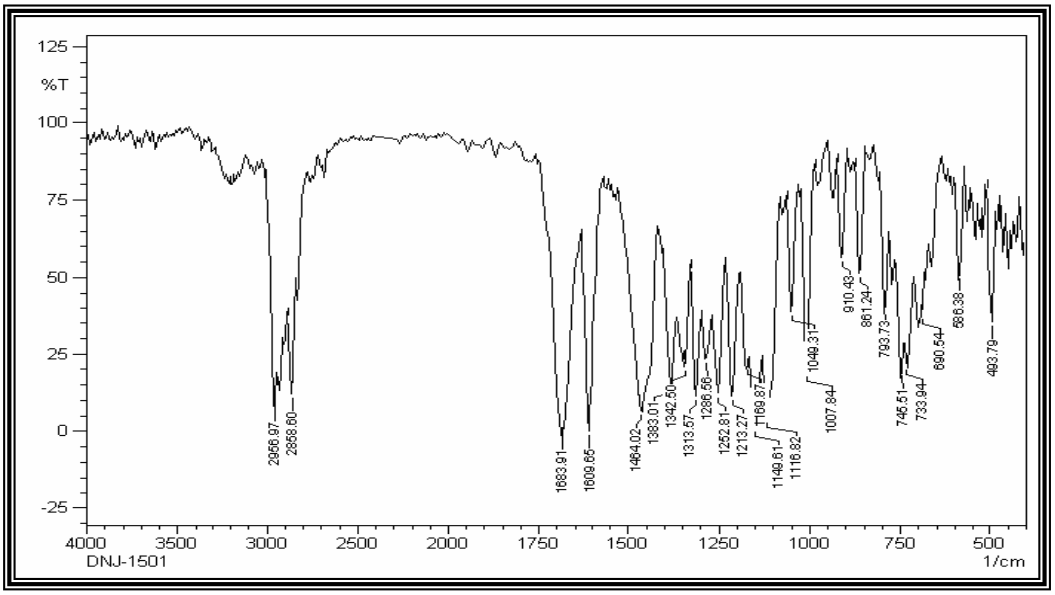
3.10.8 ^{13}C NMR Spectrum of DNJ-1401



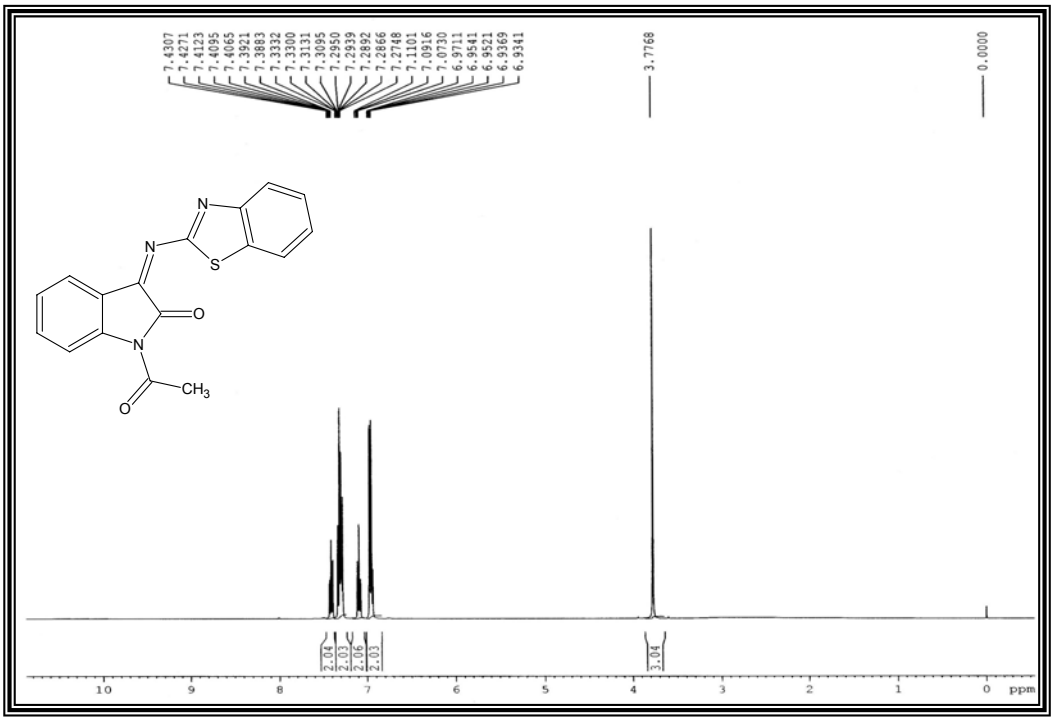
3.10.9 Mass Spectrum of DNJ-1501

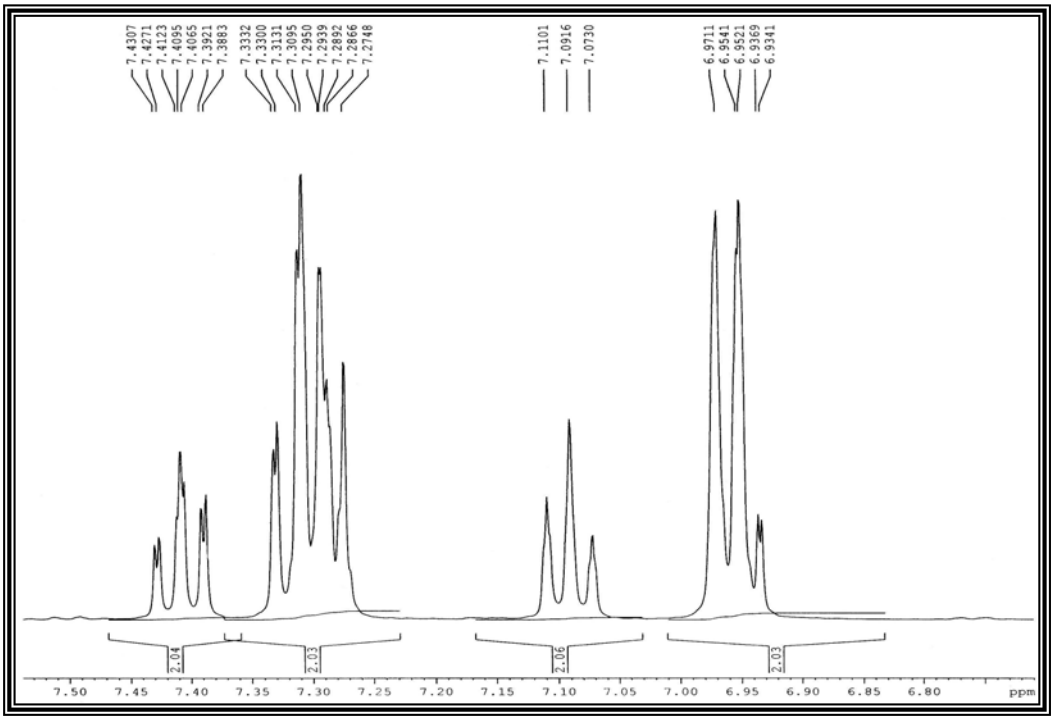


3.10.10 IR Spectrum of DNJ-1501

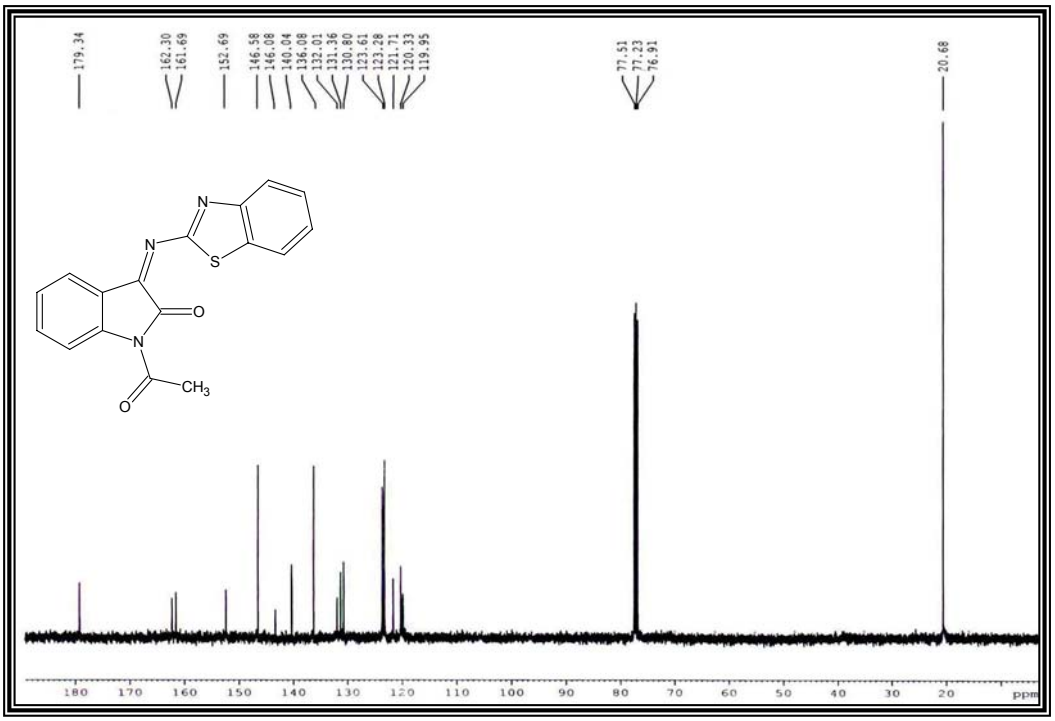


3.10.11 ^{1}H NMR Spectrums of DNJ-1501





3.10.12 ^{13}C NMR Spectrum of DNJ-1501



CHAPTER – 4

STUDIES ON DIFFERENT TYPES OF REACTIONS ON PYRAZOLE CORE STRUCTURE

4.1 INRODUCTION TO PYRAZOLE ALDEHYDES

Pyrazole refers both to the class of simple aromatic ring organic compounds of the heterocyclic series characterized by a five-membered ring structure composed of three carbon atoms and two nitrogen atoms in adjacent positions and to the unsubstituted parent compound. Being so composed and having pharmacological effects on humans, they are classified as alkaloids, although they are rare in nature.

The synthesis of pyrazoles remains of great interest owing to the wide applications in pharmaceutical and agrochemical industry due to their herbicidal, fungicidal, insecticidal, analgesic, antipyretic and anti-inflammatory properties. [1, 2] Some methods have been developed in recent years, though the most important method is the reaction between hydrazines and β-dicarbonyl compounds. [3] This reaction involves the double condensation of 1, 3-diketones or α, β-unsaturated ketones with hydrazine or its derivatives. [4, 5] However, the appealing generality of this method is somewhat vitiated by the severe reaction conditions or the multistep sequences usually required to access the starting materials. [6] Thus, continuous efforts have been devoted to the development of more general and versatile synthetic methodologies for this class of compounds. [7]

The application of Vilsmeier–Haack (VH) reagent ($POCl_3$ / DMF) for formylation of a variety of both aromatic and heteroaromatic substrates is well documented. [8] Besides this, the reagent has also been extensively used for effecting various chemical transformations from other classes of compounds. Many of these reactions have led to novel and convenient routes for the synthesis of various heterocyclic compounds. [9] A notable example that finds significant application in heterocyclic chemistry is the synthesis of 4-formylpyrazoles from the double formylation of hydrazones with Vilsmeier-Haack (VH) reagent. [10, 11] These observations, coupled with the recent developments on the simple synthesis of pyrazole derivatives, [1, 2] especially 4-functionalized 1, 3-diphenylpyrazoles as antibacterial, [12] anti-inflammatory, [13, 14] antiparasitic, [15] and antidiabetic [16] drugs, prompted

chemistry research to undertake the synthesis of pyrazole-4-carboxldehyde derivatives using Vilsmeier-Haack (VH). [17-19] The study is particularly aimed at developing a one-pot synthesis of pyrazole-4-carboxaldehyde oximes starting from acetophenone phenylhydrazones.

4.2 INTRODUCTION TO OXINDOLE

Oxindole (1, 3-dihydro-2*H*-indol-2-one; C_8H_7NO) is an aromatic heterocyclic organic compound. It has a bicyclic structure, consisting of a six-membered benzene ring fused to a five-membered nitrogen-containing ring. The compound's structure is based on the indoline structure but where a carbonyl is situated at the 2-position of the five-membered ring.

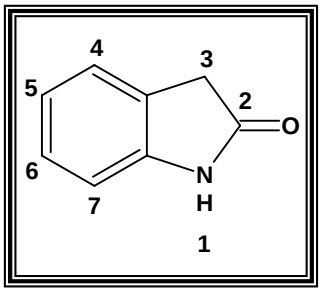


Fig. 4.1

4.2.1 PHYSICAL PROPERTIES OF OXINDOLE

Oxindole crystallizes from water in colourless needles melting at 126-127°C. The substance boils at 195°C at 17 mm [a] and at 227°C at 73 mm. [21] It is soluble in hot water, alchohol, benzene, ether and acetic acid. It is more soluble in alkaline solutions than in water. The heat of combustion at constant volume has been found to be about 950.5 kg.-cal./mole. [b]

The chemistry of indoles especially that of oxindole derivatives dates back to 134 years old when first Baeyer [20] published the results of his researches on the reaction of isatin. In addition to isatide, which had been obtained previously by Laurent [21-23] and by Erdmann, [24] Baeyer obtained dioxindole ($C_8H_7NO_2$) by the further reduction of which oxindole was prepared. Baeyer established the constitution of oxindole as the lactam of 2-aminophenylaceticacid through its synthesis by the reduction of 2-

[a] A. Waul and P. Bagard; *Compt. rend.*, **1910**, *149*, 132.

[b] M. Berthelomt and G. Andre; *Compt. rend.*, **1899**, *128*, 970.

nitrophenylaceticacid with tin and hydrochloric acid. Current practice in oxindole nomenclature is to number the positions as shown in the Fig. 4.1. Oxindole is frequently called indolinone which is now well accepted everywhere. [25, 26]

4.2.2 SYNTHESIS OF OXINDOLES

Synthesis of oxindole and its derivatives are also a part of known chemistry where many methods are reported.

Baeyer and Knop [27] found that when isatin isreduced with sodium amalgam in alkaline medium 3-hydroxy oxindole (dioxindal) is obtained. Further reduction of dioxindole with tin and mineral acids or by sodium amalgam in acid medium gave oxindole.

One convenient method for preparing oxindole is that of Marschalle. [28, 29] In this procedure isatin is reduced to dioxindole through the agency of sodium hydrosulphite. Dioxindale is then reduced to oxindole by the action of sodium amalgam in a solution saturated with carbon dioxide.

Isatin was also reduced to oxindole through the agency of hydrazide by Curtius and Thun. [30]

The first synthesis of oxindole (other than the reduction of isatin) and the one who established its constitution with certainly was by Baeyer [31] through the reduction of 2-nitrophenylaceticacid with tin and hydrochloric acid.

Reduction of 2-nitro phenylacetic acid with Zn and hydrochloric acid gives both oxindole and 1-hydroxyoxindole (1, 2-dioxindole) [32-34]

Baeyer and Comstok [35] prepared oxindole from the barium salt of 2-aminophenyl acetic acid by acidifying and then heating.

Substituted oxindoles have been prepared by the reduction of substituted derivatives of 2-nitrophenylacetic acid by Wispec, [36] Smith and Macmullen [37] and many other workers.

Oxindole was also prepared by Suida [38] through the reduction of 2-acetaminomandelic acid by either hydroiodic acid and phosphorous or sodium amalgam.

A procedure developed by Hinsberg [39, 40] serves for the preparation of *N*-alkyloxindoles. A secondary aromatic amine is condenced with the sodiumbisulphite addition compound of glyoxal; the resulting product gives an *N*-alkyl oxindole on treatment with aqueous hydrochloric acid.

Oxindole was obtained by Mazzaro and Borgo [41] by steam distillation in the presence of hydrochloric acid of the product obtained when oxindole is treated with sulfuryl chloride.

N-alkylindoles and *N*-substituted indole-α-carboxylicacids have been converted into the corresponding oxindoles by Colman [42] and Michaelis. [43]

Brunner [44-46] prepared oxindole by heating β-derivative of many substituted oxindoles especially 3, 3-acetylphenylhydrazine with lime at 200-220°C this procedure has been extended by Brunner [47] and by others [48-52] to the preparation dialkylderivatives of oxindole.

Another convenient and general method for the preparation of oxindole and of *N*-substituted oxindoles is that of Stolle. [53-57] An α-halogenated acid chloride or bromide is condensed with an aromatic amine. Subsequent ring closure with aluminiumchloride yields the corresponding oxindole.

In the course of the investigation of the structure of physostigmine (esertine) Stedman and Barger [58] obtained the oxindole derivatives as a degradation product. Catalytic reduction of oxindole derivative gives physostigmine.

6-aminooxindole was prepared by Gabrial and Meyer [59] by the reduction of 2, 4-dinitrophenylaceticacid with tin and hydrochloric acid.

o-aminophenylaceticacid condensed with o-nitrobenzaldehyde to give what Neber thought was quinoline derivative, but the product was shown by Kliegl and Schmalenback [60] to be 3-(o-nitrobenzal) oxindole.

Recently many workers have reported direct or indirect synthesis of oxindole [61-100] and 2, 6-dichlorophenyl-2-indolinone [101-105] using different methodologies.

4.2.3 SYNTHETIC OXINDOLES AS ENZYME INHIBITORS

A new series of isatin derivatives was designed, by utilizing a combination of protein structure based drug design, molecular modelling, and structure activity relationship (SAR). These compounds were subsequently synthesized and evaluated as inhibitors of human coronavirus 3C-like and rhinovirus proteases. [106] Modelling studies of these protein-inhibitor complexes lead to some interesting conclusions about placing specific groups at N_1 and C_5 position. The C_2 carbonyl of isatin was envisioned to react in the active site of human rhinovirus (HRV) 3C protease with the cysteine responsible for catalytic proteolysis, thus forming a stabilized transition state mimic. (Fig. 4.2)

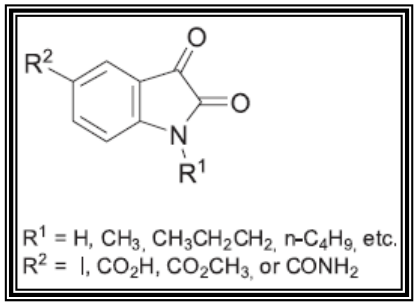


Fig. 4.2

More recently, a series of novel oxindoles were discovered as efficient HIV (human immunodeficiency virus) non-nucleoside reverse transcriptase inhibitors. Systematic structural modifications of the lead molecule defined its SAR, and permitted the identification of increased antiviral activity at the early stages in infection. [107] These studies suggested that both the NH and the carbonyl oxygen moieties are crucial for the antiviral activities, and also

demonstrated that the cyclopropane moiety is critical in optimizing effective molecular interactions. (Fig. 4.3)

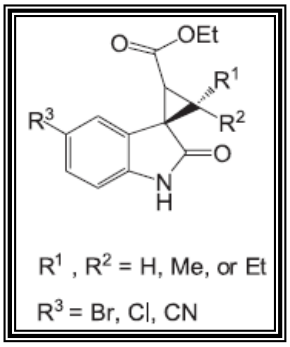


Fig. 4.3

2-Indolinone derivatives have recently been established as anti cancer compounds [108] and more specifically as tyrosine kinase inhibitors (*SU5416*, *SU5614*, *SU6668*, *SU6597*, *SU6663* and *SU6561*-Fig. 4.4) that block kit activation and growth of small cell in lung cancer. [109] Targeting receptor kinase by novel indolinone derivatives like BIBF1000 in multiple myeloma abrogration of stroma derived interleukin-6 secretion and induction of apoptosis in cytogenetically with definite subgroups has been identified. [110] Various other analogs have been evaluated as kinase inhibitors, anticancer and antiangiogenic agents with encouraging results. [111]

After the first report of fibroblast growth factor receptor (FGFr-1) co-crystallization with *SU4984* and *SU5402*, the crystal structure of a receptor tyrosine kinase (RTK) bound to a competitive inhibitor of ATP was identified. [112] These compounds are also inhibitors of both the vascular endothelial growth factor receptor (TK flk-1) and vascular endothelial growth factor (VEGF). *SU4984* and *SU5402* inhibit the autophosphorylation of FGFr with IC_{50} values of 10-40 μM. [113]

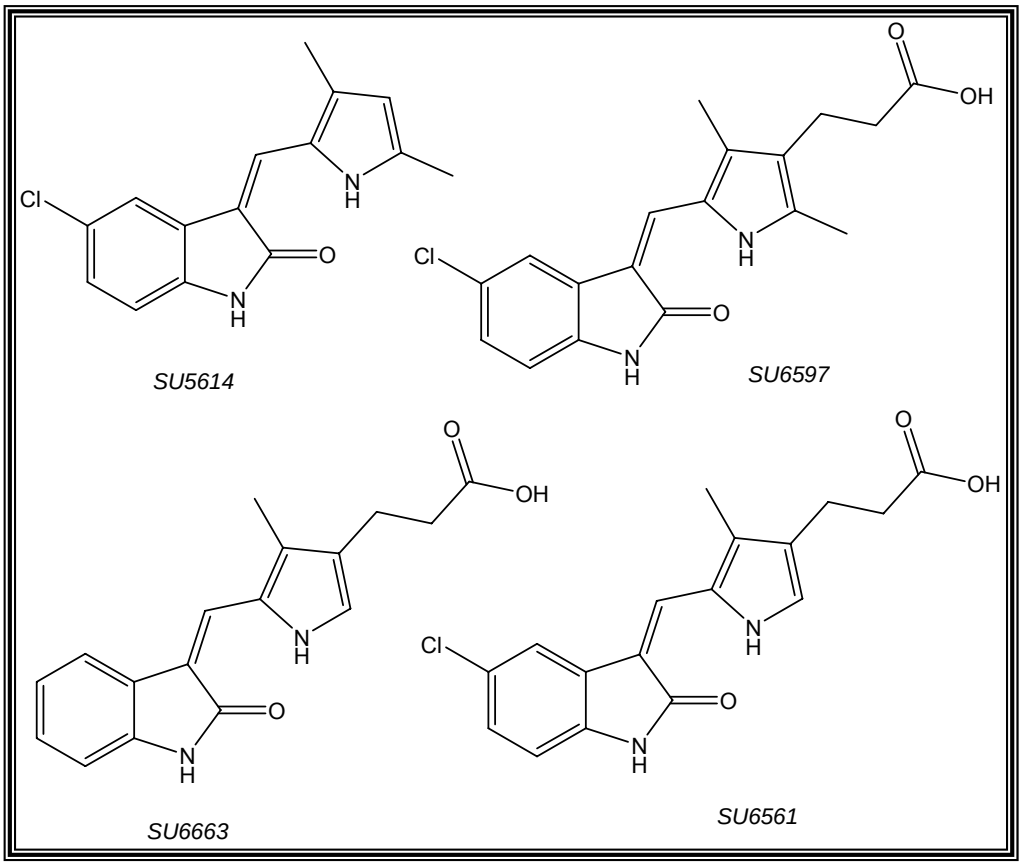


Fig. 4.4

SU11248 is designed to bind in particular, VEGF receptor, PDGF receptors α and β, Flt3 and C-KIT tyrosine kinases. [114, 115] The other derivatives studied are *SU6577* and *SU6663*.

All molecules of *SU* series are indolines, unsubstituted at the nitrogen of the indole ring, while the molecules developed by Boehringer Ingelheim Pharma, Ingelheim (DE) are oncolytic and various tyrosine kinase receptor inhibitors and similar to the Sugen molecules, but substituted in the 6-position of the indolinone nucleus. [116] Recently Andreani et al. [117] extensively studied several *E* isomers of 3-(3, 4, 5-trimethoxybenzylidene)-1, 3-dihydroindol-2-ones as anticancer agents. Moreover, (2-chloroindolyl) methylene-2-indolinone derivatives were studied as CDK1/cyclinB inhibitors by the same author. [118] The closely structurally related compounds 3-(((4-phenyl)-piperazine-1-yl)-alkyl)-3-alkyl-1,3-dihydro-2*H*-indol-2-one derivatives and

related compounds have shown as CNS antagonists which bind 5-HT_2C and a1 receptors. [119]

Further, these indolinone-derivatives were prepared by attaching different chemical substituents to an oxindole-core, and were observed to act as efficient inhibitors of protein tyrosinase kinases (PTKs). [112] These protein kinases are critical components of signaling pathways in the control of cell proliferation and differentiation, and enhanced PTK activity has been implicated in many human cancers. [120] Thus, selective inhibitors of this class of proteins can have considerable therapeutic value, as compounds *SU4984* and *SU5402*. Some oxindole and aza-oxindoles were synthesized and described as potent inhibitors of TrkA tyrosine kinases, a subclass in this family of enzymes, such as compounds A and B. Some substituents with similar scaffold in these compounds can increase the selectivity for TrkA inhibition over CDK2, another class of kinase proteins. As an example, compound A showed IC_{50} = 0.008 μmol L^{-1} for TrkA, and 10.4 μmol L^{-1} for CDK2. [121] (Fig. 4.5)

Another compound with similar structure, *SU9516*, a novel 3-substituted indolinone compound, had effects on colon cancer cell kinase activity, cell proliferation, cell cycle progression, and apoptosis examined. [122] In this case, the studies aimed at the so called cyclin-dependent kinases (CDK), which are key-regulators of the cell cycle. Protein kinases constitute a large class of proteins that catalyze the phosphorylation of target proteins and enzymes. They can regulate a wide range of processes including carbohydrate and lipid metabolism, neurotransmitter biosynthesis, DNA transcription and replication, organelle trafficking, smooth muscle contraction, and cell differentiation. [123] These proteins are specific for each phase of the cell cycle, and determine the progression of distinctive and well ordered phosphorylation events occurring. Therefore, they constitute promising target for anticancer drug development. [124] It was demonstrated that the oxindole *SU9516* is a potent and selective inhibitor of the CDK2 kinase catalytic activity; it also decreases ligand -dependent and -independent cell cycle progression, and increases apoptosis in a cell line-specific manner.

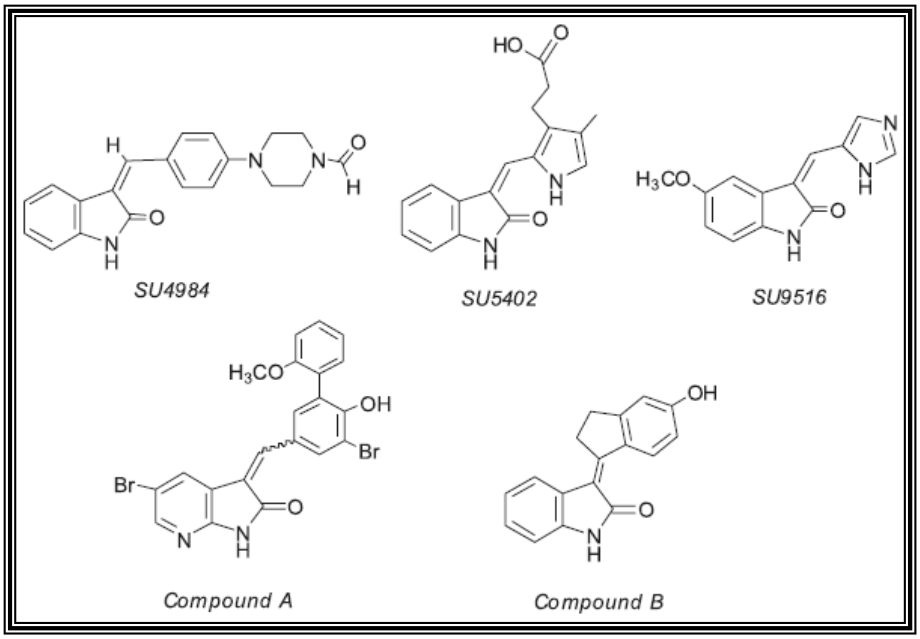


Fig. 4.5

On the other hand, some oxindole-derivative compounds have been reported as potent inhibitors of vascular endothelial growth factor (VEGF) that stimulated angiogenesis. [125] Angiogenesis, the development of the microvasculature, is a complex multistep event, described as a crucial aspect of tumor growth and spread. Tumors with greatest vasculature are often associated with poorer prognosis and higher metastatic potential. Angiogenesis inhibitors prevent the growth of blood vessels from surrounding tissue of a solid tumor, and those VEGF exert their effect in tumor growth by binding to cell surface receptors which have intrinsic tyrosinase kinase activity. [126] The oxindole derivatives named *SU5416* and *SU6668* inhibited angiogenesis in vitro by >86% at 10 μmol L^{-1} concentration, and this inhibition was already significant at 1 μmol L^{-1}. Both compounds are now a days in clinical trial for cancer treatment. [127] (Fig. 4.6)

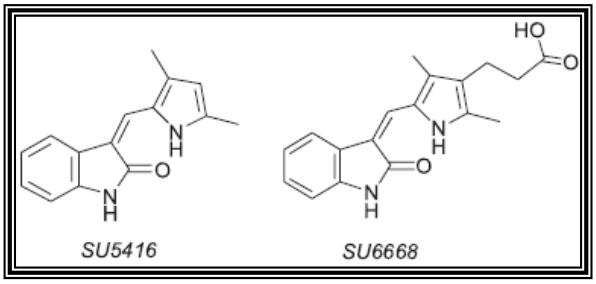


Fig. 4.6

Novel oxime-derivatives of indirubin, a metabolite of isatin oxidation and an active ingredient of a traditional Chinese medicine containing a bis-oxindole skeleton have been recently developed, based on structure-activity relationship studies, as potent cancer cell growth inhibitors for different cell lines. Recent investigations indicated that these oxindoles display high inhibition of some cyclin-dependent kinases (CDK2). [128] (Fig. 4.7)

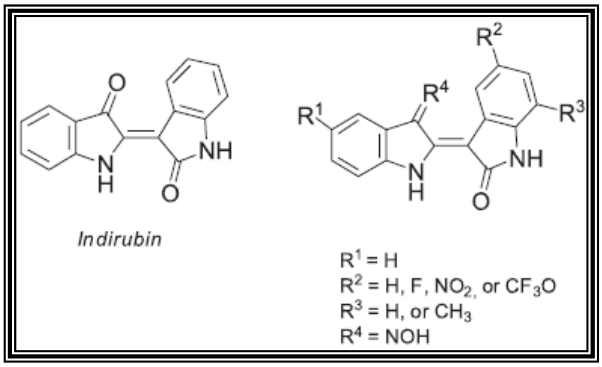


Fig. 4.7

Moreover recently Virsodia *et. al.* [129] reported *in vitro* anticancer evaluation of 1-(2, 6-dichlorophenyl)-3-methylene-1, 3-dihydro indole-2-one derivatives against SW 620 colon cancer cell line. (Fig. 4.8)

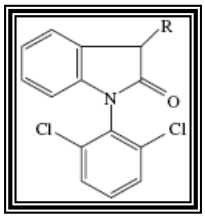


Fig. 4.8

Where R = Differently substituted benzaldehydes

4.3 INTRODUCTION TO COUMARIN

Coumarin is a chemical compound (2*H*-chromen-2-one, 1-benzopyran-2-one, benzopyrone; $C_9H_6O_2$); a toxin found in many plants, notably in high concentration in the tonka bean, vanilla grass, woodruff, mullein, and bison grass. It has a sweet scent, readily recognised as the scent of newly-mown hay, and has been used in perfumes since 1882. It has clinical medical value as the precursor for several anticoagulants, notably warfarin, and is used as a gain medium in some dye lasers.

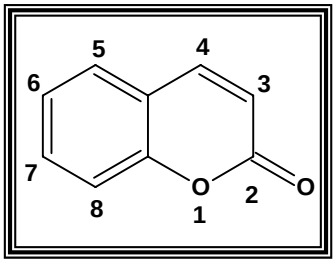


Fig. 4.9

The isolation of coumarin was first reported by Vogel [130] in 1820. He isolated coumarin from tonka beans, bearing the characteristic aroma of cutted grass. The name of coumarin originates [131] from a Caribbean word "*coumarou"* for the tonka tree, which was known botanically at one time as *Coumarouna odorta Aubl*, coumarin is now the accepted trival name. Coumarin was first synthesized in 1868 on treatment of sodium salt of o-hyroxy benzaldehyde with acetic anhydride. [132a, 132b] Compounds containing coumarin subunit possess a wide range of activities and show an interesting reactivity. [133-136] This is consequence of the rich electronic structure of coumarin which offers abundant possibilities for diversified activity and reactivity of the system.

4.3.1 SYNTHESIS OF 4-HYDROXY COUMARIN

Perkin [132] synthesized coumarin and then several methods are reported for the synthesis of 4-hydroxy coumarins and their 4-hydroxy substituted derivatives namely:

1 Anschutz method [137]
2 Pauli Lockemann synthesis [138]
3 Sonn's synthesis [139]
4 Mentzer's synthesis [140]
5 Robertson synthesis [141]
6 Ziegler and Junek method [142]
7 Garden's method [143]
8 Shah, Bose and Shah's method [144]
9 Kaneyuki method [145]
10 Resplandy's method [146]
11 Jain, Rohatagi and Sheshadri's method [147]
12 Shah, Bhatt and Thakor's method [148]

Shah and co-workers [144, 148] have prepared 4-hydroxy coumarin derivatives in good yield by condentation of different phenols with malonic acid in the presence of zinc chloride and phosphorous oxychloride. The method is useful as single step preparation of 4-hydroxy coumarin derivatives substituted in benzenoid part.

Recently many researchers [149-180] have reported synthetic strategies for 4-hydroxy coumarin.

4.3.2 BIOLOGICAL ACTIVITIES ASSOCIATED WITH 4-HYDROXYCOUMARIN DERIVATIVES

Numerous biological activities have been associated with simple coumarins and its analogues. Among them, antimicrobial, antiviral, anticancer, enzyme inhibition, anti-inflammatory, antioxidant, anticoagulant and effect on central nervous system are most prominent. Coumarin nucleus possesses diversified biological activities that can be briefly summarized as under:

1 Antimicrobial and Molluscicidal [181-202]
2 Antiviral [203-207]
3 Anticancer [208-218]
4 As Enzyme Inhibition [219-224]

5 Antioxidant [225-228]

6 Anti-inflammatory [229-233]

7 Anticoagulant and Cardiovascular [234-237]

8 Effect on Central Nervous System [238-240]

4-hydroxycoumarin is a versatile scaffold and is being consistently used as a building block in organic chemistry as well as in heterocyclic chemistry for the synthesis of different heterocycles. The synthetic versatility of 4-hydroxycoumarin has led to the extensive use of this compound in organic synthesis. 4-hydroxy coumarin shows diversified chemical reactivity. Preparation of 3-acetyl-4-hydroxycoumarinyl chalcones, condensation reaction of 4-hydroxycoumarin with aldehydes and use of 4-hydroxycoumarin as a β-keto ester in dihydropyrimidine synthesis have been discussed herein.

4.3.3 INTRODUCTION TO COUMARINYL CHALCONES

The compound 1, 3–diphenyl-2-propene-1-one is known by its trivial name chalcone. Many naturally occurring flavonoids share structural features with chalcone and are referred by the generic term “chalcones”. This is well illustrated by benzal-acetophenone or phenyl styryl ketone or phenyl acreloacetophenone named first time as chalcones by Kostanecki and Tambor. [241] Certain flavonoids also include chalcone subunit and found to have anticancer activity and chemopreventive activity in some tumors.

Additionally, some natural or synthetic chalcones are proved to have significant antiproliferation activity on different cell lines. Though the mechanism of antiproliferative activity of flavonoids and chalcones is still unknown, it is believed to be linked with the interaction of type II estrogen receptors.

The *in vivo* action of these polyphenol substances is certainly much more complicated. All these compounds are generally characterized by an almost complete solubility in water and, *in vivo*, by a very poor bioavailability linked to a rapid metabolism of phenols and a marked affinity for lipids and

proteins. It has now been found that certain novel chalcones, chalcone derivatives and chalcone analogues, in particular ones in which the phenyl ring at the 1st position is substituted or replaced by the rings containing one or more heteroatoms, possess greater antiproliferation activity both on sensitive cancer cells or on cells which are resistant to common chemotherapeutic drugs, including the latest generation of anti-neoplastic agents, pacitaxel and docetaxel.

In recent patent literature, compounds of the general formula shown in Fig. 4.10 are a pharmaceutically acceptable salt or solvate there of wherein Ar represents a substituted or unsubstituted, (preferably aromatic), carbocycilc or heterocyclic group, said carbocyclic or heterocyclic group containing from 5 to 10 ring atoms, said ring atoms forming one or two rings, wherein each ring contains 5 or 6 ring atoms, any heteroatoms being selected from N, O and S, any substituents on the Ar group being independently selected from the group consisting of: (a) Cl, (b) Br, (c) F, (d) OH, (e) NO_2, (f) CF_3, (g) C_{1-4} lower alkyl (in particular CH_3), (h) SCH_3, (i) $NHCOCH_3$, (j) $N(R)(R_1)$ wherein R and R_1 are the same or different and each represents H or lower C_{1-4} alkyl. (Fig. 4.10, 4.11, 4.12 and 4.13)

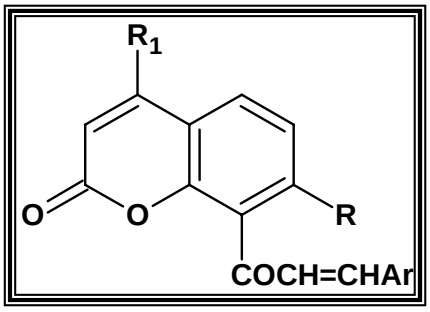


Fig. 4.10

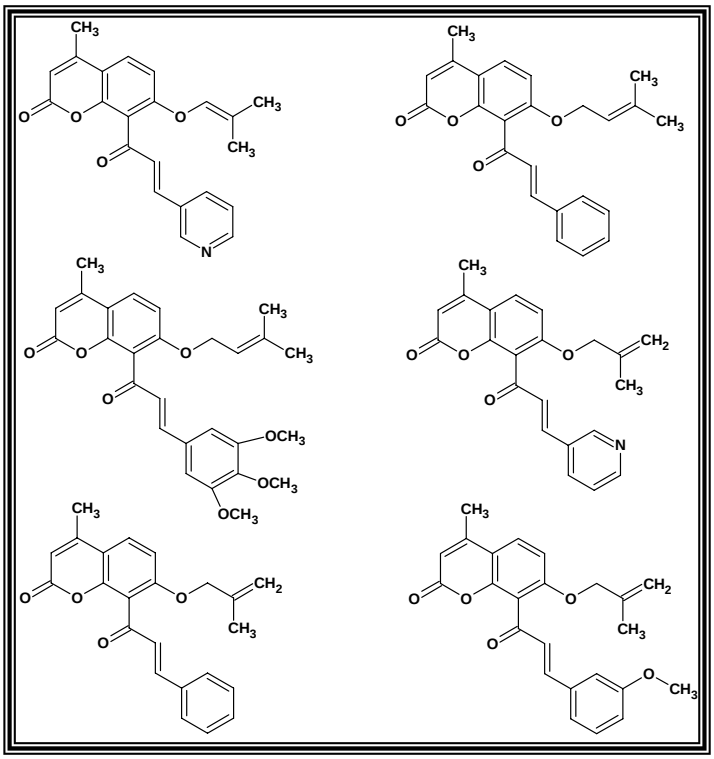


Fig. 4.11

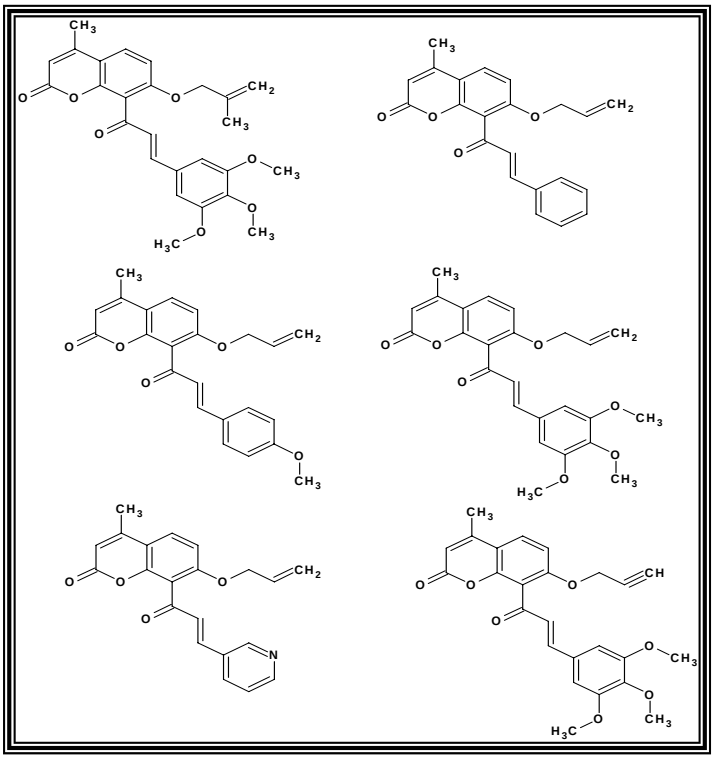


Fig. 4.12

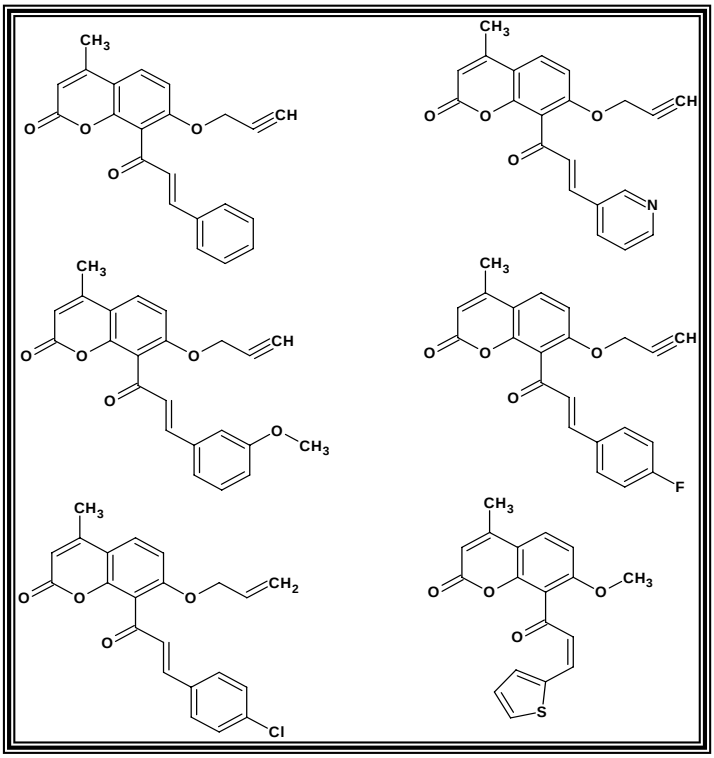


Fig. 4.13

Several 2'-hydroxy chalcones are found to exist as pigments. The natural chalcones are found to contain phloroglucinol, pyraogallol, catechol and hydroquinone nuclei. [242-246]

Chalcones contain keto-ethylenic linkage and therefore reactive towards reagents like phenyl hydrazine, hydrazine hydrate and ethyl acetoacetate to produce heterocyclic derivatives. Chalcones have close relationship to flavones, flavanones, flavanols and dihydroflavanols. They are useful as intermediates in the synthesis of certain heterocyclic compounds like flavones, anthocyanins and benzal coumarones. [247-250] Butein, phloretin and hissopin are found to be naturally occurring chalcones. Sometimes, chalcones are found to occur in nature as glycosides like carthamin and isocarthamin present in *Carthamus tinctorious*. [251]

2'-Hydroxy chalcones are used as starting material to synthesize naturally occurring flavanones, flavones, flavonols, etc. The chalcones are also natural biocides [252-254] and are well known intermediates in the synthesis of heterocyclic compounds exhibiting various biological activities like

antimalarial, [255] antiviral, [256] antitumor, [257] herbicidal [258] and also bactericidal [259, 260] activities. They are also identified as antioxidants. [261]

Curcumin is a yellow pigment isolated from the rhizome of the perennial herb *Curcuma longa L* (turmeric). The chemical structure of curcumin was elucidated by Lampe *et. al.* [262]

Curcumin has several biological activities. It possesses anti-inflammatory, antioxidant, antibacterial, antihepatotoxic, hypotensive and hypocholesterolemic properties. [263-266] Tonneses [267] describes curcumin as a non-toxic compound even at high dosages. It has a dual effect in oxygen radical reactions, thus it can act as a scavenger of hydroxyl radicals or catalyse the formation of hydroxyl radicals depending on the experimental conditions. [267, 268]

Curcumin inhibits *in vitro* lipid peroxide formation by liver homogenates of oedemic mice. [269] The inflammatory response induced experimentally in animals appeared to be corelated with disturbances of the regulation of cellular oxidative process, as is evident from the anti-inflammatory action of well known antioxidants. There is evidence of a parallel between the inhibition of aedema formation in mice induced by carrageenan and the decrease in the production of lipid peroxides in liver homogenate. [269] Modification of groups on the terminal aromatic rings of curcumin reveals that electron donating groups increase anti-inflammatory activity. [270] The structural similarity of chalcone like molecules is expected to exhibit either antagonize or potentiate the biological activity in question and therefore it was very essential to study further, the coumarin derivatives possessing such ethylenic linkages-discussed earlier.

4.3.4 CHALCONES OF 3-ACETYL-4-HYDROXYCOUMARIN

4-hydroxycoumarin is known to give 3-substituted-4-hydroxycoumarin derivatives on electrophilic or nucleophilic attack; acetylation of 4-hydroxycoumarin using glacial acetic acid in presence of phosphorous oxychloride to yield 3-acetyl-4-hydroxycoumarin is one of it. Subsequent

reaction of 3-acetyl-4-hydroxycoumarin with aldehydes forms chalcone at C_3 position with the elimination of water molecule under basic condition. These chalcones are excellent intermediates to build different heterocycles by means of further cyclization using it.

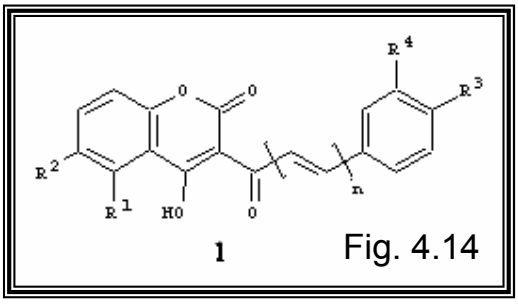
Recently coumarinyl chalcones have drawn attention of chemistry researchers towards it chemistry and antiviral activity. 4-hydroxycoumarinyl chalcones were prepared from 3-acetyl-4-hydroxycoumarins via Claisen condensation with benzaldehydes by Mulwad *et. al.*, [271] which were further cyclized on treatment with 10 % sulfuric acid in ethanol and selenium dioxide in amyl alchohol to give 4*H*, 5*H*-2, 3-dihydro-2-phenylpyrano [3, 2 - c] – 1 – benzopyran - 4, 5 - diones and 4*H*, 5*H* – 2 – phenylpyrano [3, 2 - c] – 1 – benzopyran - 4, 5 – diones respectively. The chalcone oximes on refluxing with silica gel and dichloroethane gave 2 – oxo – 3 – styryl - 4*H* – 1 – benzopyran [3, 4 - d] isoxazoles.

Due to the exceptional reactivity of 4-hydroxycoumarin and the versatile biological activity of coumarin derivatives, the synthesis of 3-substituted 4-hydroxycoumarin derivatives was carried out and tested for antibacterial and antifungal activity by Zavrsnik *et. al.* [272] Microbiological activity of synthesized compounds was executed using diffusion method on the species of bacteria *Escherichia coli* 113-1, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* SG 511, and fungi *Candida albicans* ATCC 10231. The antibacterial activity of currently used antibiotics was also carried out on the same species of bacteria for comparison point of view. The synthesis of derivatives of 3-cinnamoyl-4-hydroxycoumarin and azomethines of 4-hydroxycoumarin was also carried out. Both the groups of compounds showed significant microbiological activity. All synthesized compounds showed antimycotic and antibacterial activity. Derivatives of 3-cinnamoyl-4-hydroxycoumarin in relation to azomethines of 4-hydroxycoumarin showed better activity in case of all the bacteria and fungi species. The best antibacterial activity was shown by the species which had -Cl or $-OCH_3$ as substituents, and the best antimycotic activity was shown by the species which contained Cl.

Preparation of 4-hydroxycoumarin, its 3-acetyl derivative was carried out by Mohamed *et. al.* [273] Further this 3-acetyl-4-hydroxycoumarin was condensed with aryl aldehydes to afford respective α-hydroxychalcones. They have also reported further cyclization of chalcones into different heterocyclic moieties.

3-acetyl-4-hydroxylcoumarin was synthesized by acetylation and acyl chlorination of salicylic acid followed by the cyclocondensation of acetoacetic ester, then condensation with aldehydes to obtain the three title product by Ding *et. al.* [274] The three derivatives have high extinction coefficients in UV-vis absorption, which were 5.25 x 104 L/(mol-cm), 3.39 x 104 L/(mol-cm), and 3.24 x 104 L/(mol-cm), respectively, and intensive luminescence in both the THF solution and solid state. Derivatives TM1 and TM2 emit intensive orange red luminescence with the same absorption peak at 500 nm and the identical small Stoke shift of 85 nm in the emission spectra in THF. The photoluminescence peaks of the three derivatives in solid state are at 705, 643, and 660 nm, respectively. Derivative TM3 displays intensive red luminescence with the peak at 486 nm and a large Stoke shift of 152 nm in the emission spectrum in THF, which makes it a novel red luminescent material that may be used in OLEDs.

Title compounds (1) [R^1 = H, OH; R^2 = H, OH, OMe, F, Cl; R^3 = H, OH, OMe, F, CF_3, Cl; R^4 = H, OMe; n = 1, 2] were prepared for use as HIV-1 protease inhibitors by Kulkarni *et. al.* [275] Thus, 4-hydroxycoumarin was treated with PhCH=CHCOCl to give (1) [R^1-R^4 = H, n = 1] which had ki for HIV-1 protease inhibition of 10 μM. (2*Z*, 4*E*)-5-phenylpenta-2, 4-dienoic acid was prepared by treating 3-phenylacrylaldehyde and acetic anhydride which were treated with 4-hydroxycoumarin to give (1) [R^1-R^4 = H, n =2]. (Fig. 4.14)



1 Fig. 4.14

A simple synthesis and biological properties of 1, 3-diphenyl-2-propen-1-ones are described by Jang *et. al.* [276] The key synthetic strategies involve Grignard reaction of aldehyde and oxidation reaction in high yields. The prepared compounds were evaluated for free-radical scavenging, suppression of LPS-induced NO generation, and anti-excitotoxicity *in vitro*. It was found that a couple of compounds were potent suppressors of NO generation and demonstrated anti-excitotoxicity with the concentration range 10-20 μM *in vitro*.

Trivedi *et. al.* [277] synthesized differently substituted 4-hydroxy coumarins and its 3-acetyl derivatives. Further they condensed 3-acetyl-4-hydroxy coumarin with differently substituted aromatic benzaldehydes to afford coumarinyl chalcones. They not only improved the synthetic methodology of chalcones from strong alkali and alchohol to piperidine and chloroform but they reported their antiviral activity too.

4.4 INTRODUCTION ON CHROMANE DIONES

Tautomeric form of 4-hydroxycoumarin was first established by J. Kolsa. [278] He proposed that if 4-hydroxy coumarin is a 2, 4-dioxochroman than its $-COCH_2CO$ group should be reactive and easily condense with ester but show no reaction with diethylcarbonate, ethylcyanoacetate, methylacetoacetate, ethylacetoacetate and benzylacetoacetate in presence of sodium and sodium ethoxide. Thus it was concluded that 4-hydroxycoumarin enolises at the lactone carbonyl and exists mainly in coumarin tautomerism in which the enol form of coumarin is favored. (Fig. 4.15)

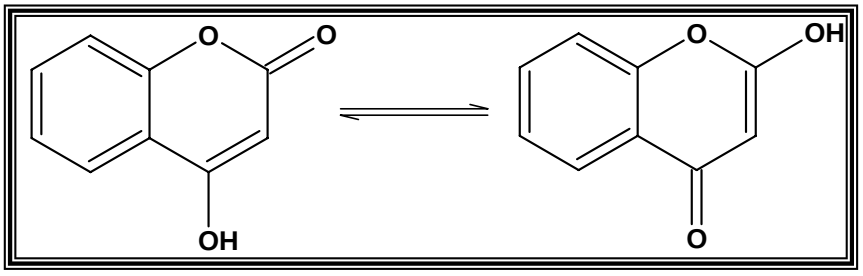


Fig. 4.15

Various methods are utilized to prepare 2, 4-chromandiones and 2, 3, 4-chromantriones shows ketonic nature of 4-hydroxycoumarin and therefore they were thought of interest to investigate their chemical reactivity and other properties. So far, 2, 4-chromandiones have been isolated as crystalline solids. Solid derivatives of 2, 3, 4-chromantriones have been reported usually as derivatives of the 3-keto group.

Extensive work has been reported on 2, 4-chromandiones and 2, 3, 4-chromanetriones. They are regarded as analogues of 4-chromanones and 3, 4-chromanediones respectively. [279] (Fig. 4.16)

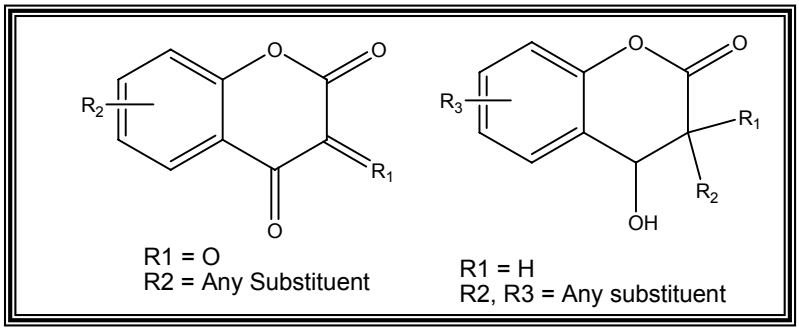


Fig. 4.16

A brief survey of literature on some 2, 4-chromandione and 2, 3, 4-chromantrione derivatives is summarized.

4-hydroxycoumarins frequently react with aromatic aldehydes to give 3-benzylidene-2, 4-chromandiones. [280-283] (Fig. 4.17)

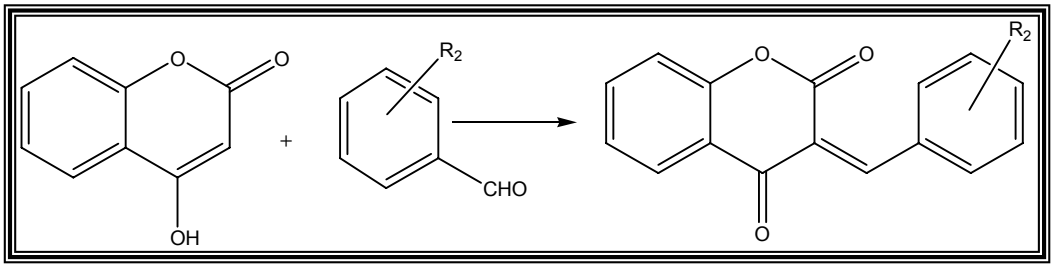


Fig. 4.17

However, reactions using salicylaldehyde or its analogues multicyclic compounds were obtained either solely or in addition to salicylidene derivatives of type as shown in Fig. 4.18. [284]

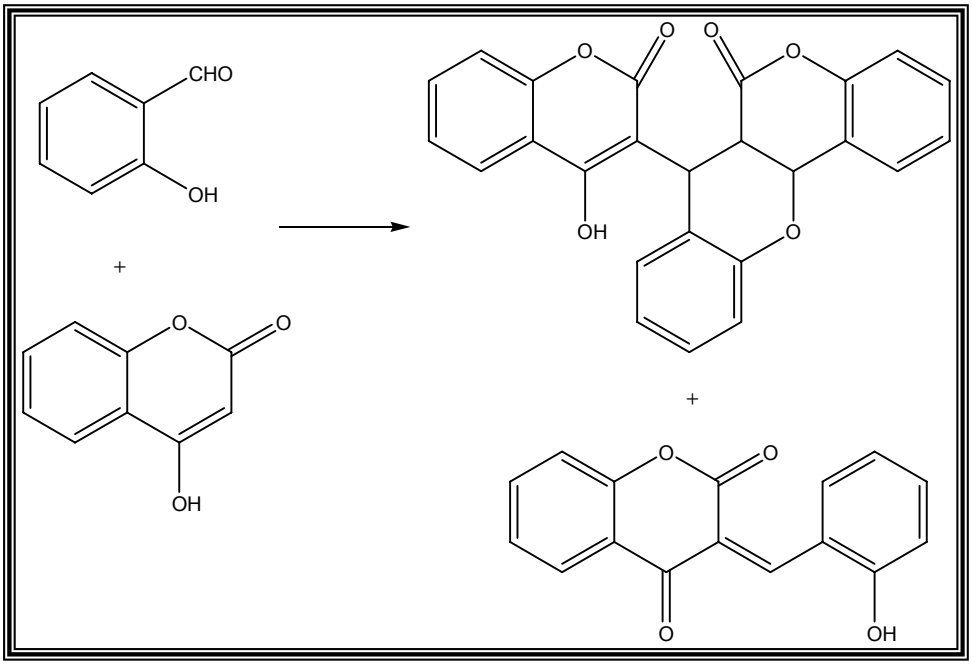


Fig. 4.18

The proportions of the products were dependent on reaction conditions used e.g. when salicylaldehyde and 4-hydroxycoumarin refluxed in ethanol; a dimeric type of structure in addition to benzylidene derivative was obtained. [285]

When two moles of salicylaldehyde were reacted with 4-hydroxycoumarins, it gave appropriate benzylidene derivative only (Fig. 4.19). However, one mole of salicylaldehyde with two moles of 4-hydroxycoumarin gave the dicoumarinyl structure. [286] (Fig. 4.20)

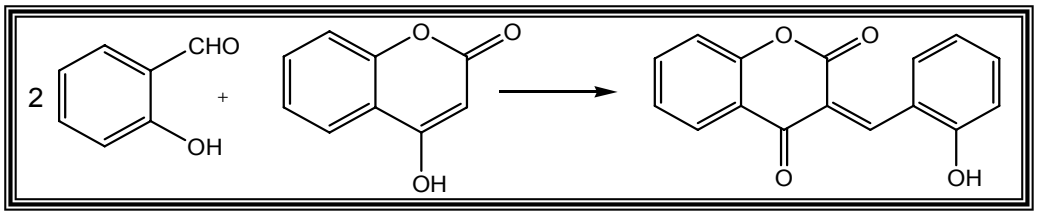


Fig. 4.19

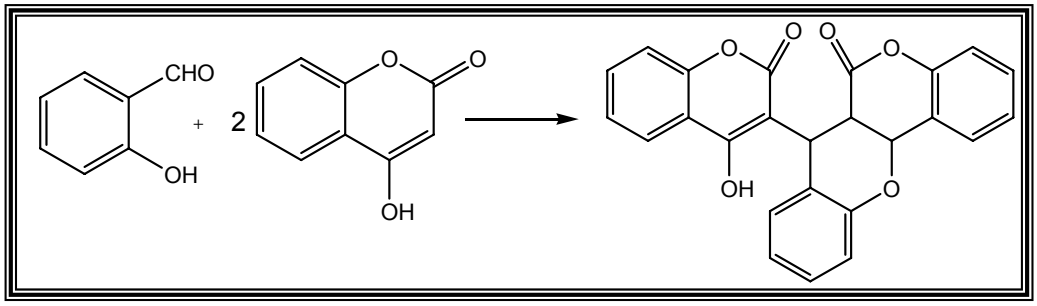


Fig. 4.20

Similarly, reaction of 4-hydroxycoumarin with acetylated aldehydohexoses in ethanol for 24 hours gave substance shown in Fig. 4.21. [287]

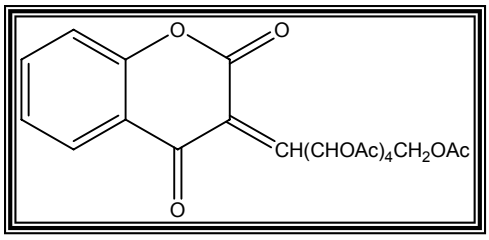


Fig. 4.21

Reaction between 4-hydroxycoumarin and hydroxylamine hydrochloride gave corresponding 2, 4-chromadione-4-oximes. [288] (Fig. 4.22)

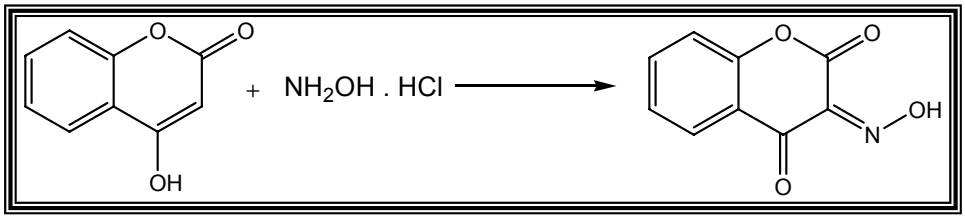


Fig. 4.22

Reaction of chlorine with 4-hydroxycoumarins in suitable solvent or sulfuryl chloride led to the formation 3, 3-dichloro-2, 4-chromandiones. [289-293] Halogenations of 3-substituted 4-hydroxycoumarin afforded 3-chloro-2, 4-chromandiones. When 3, 3' methylenebis (4-hydroxycoumarin) was treated with sufuryl chloride, 3, 3' methylenebis (3-chloro-2, 4-chromandione) was isolated. (Fig. 4.23)

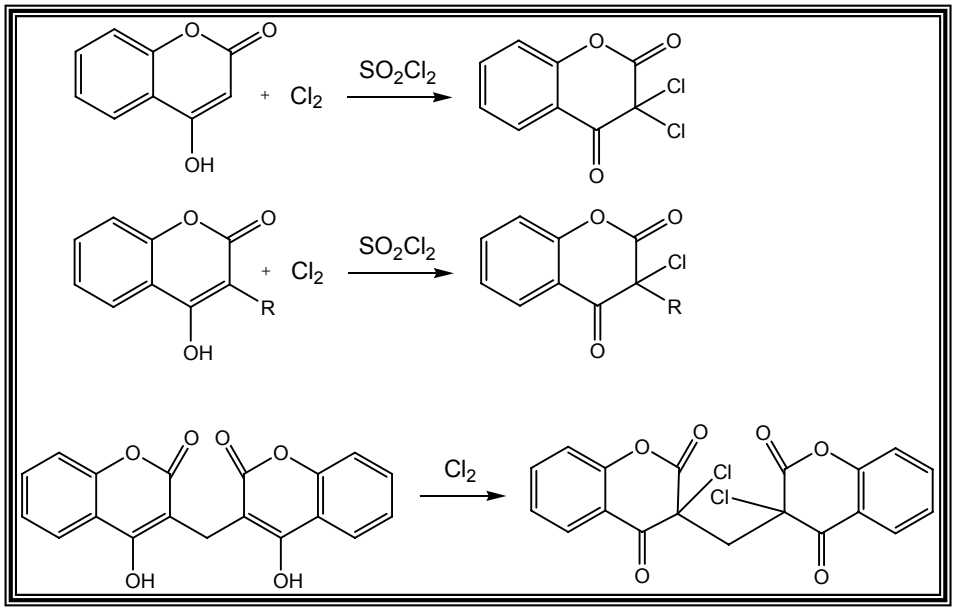


Fig. 4.23

When 3-amino-4-hydroxycoumarin was reacted with nitrous acid, it gave 3-diazo-2, 4-chromandiones. The same product was also obtained in 72% yield when sodium nitrite in dilute hydrochloric acid was added to 3-amino-4-hydroxycoumarin. [294] (Fig. 4.24)

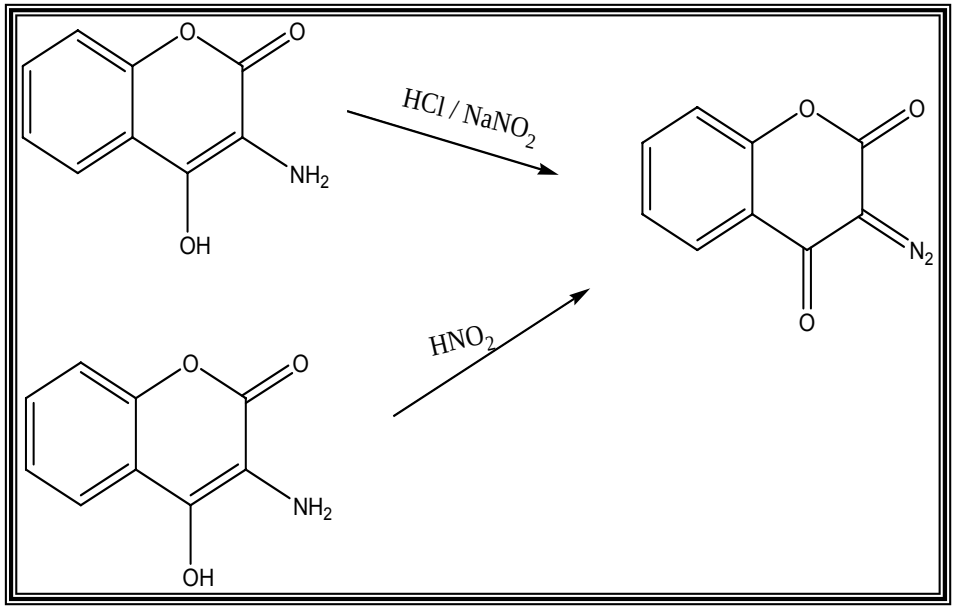


Fig. 4.24

However, reaction of 4-hydroxycoumarin with aqueous sodium nitrite afforded 2, 3, 4-chromantrione-3-oxime which forms a silver salt. [295] (Fig. 4.25)

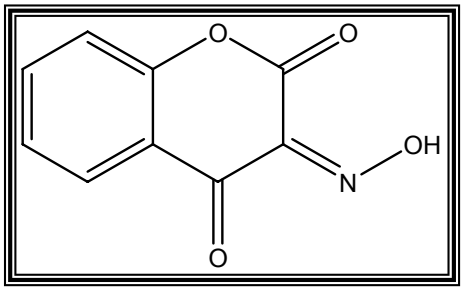


Fig. 4.25

4.5 USE OF 4-HYDROXYCOUMARIN IN DIHYDROPYRIMIDINE SYNTHESIS

Three component Biginelli reaction is very well known for the synthesis of dihydropyrimidine derivatives. P. Biginelli reported the synthesis of functionalized 3, 4-dihydropyrimidine-(1*H*)-ones (DHPMs) via three component condensation reaction of an aromatic aldehydes, urea and ethyl acetoacetate. In the past decade, this long-neglected multicomponent reaction has experienced a remarkable revival, mainly due to the interesting pharmacological properties associated with this dihydropyrimidine scaffold. [296]

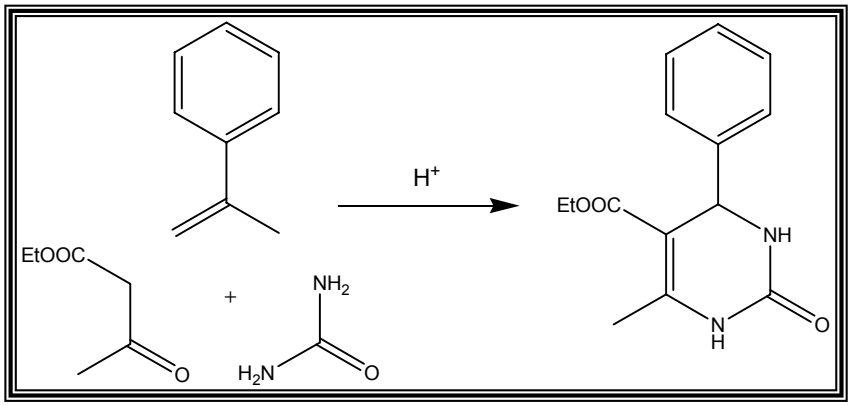


Fig. 4.26

The reaction was carried out by simply heating a mixture of the three components dissolved in ethanol with a catalytic amount of concentrated

hydrochloric acid at reflux temperature (Fig. 4.26). The product of this novel one-pot, three-component synthesis that precipitated on cooling of the reaction mixture was identified correctly by Biginelli as 3, 4-dihydropyrimidine-2(1*H*)-one. Apart from a series of publications by the late Karl Folkers in the mid 1930s, the "Biginelli reaction" or "Biginelli condensation" as it was henceforth called was largely ignored in the early part of the 20th century. The synthetic potential of this new heterocycle synthesis therefore remained unexplored for quite some time. In the 1970s and 1980s, interest slowly increased and the scope of the original cyclocondensation reaction was gradually extended by variation of all three building blocks, allowing access to a large number of multifunctionalized dihydropyrimidines. [297-299]

Dihydropyrimidinone derivatives are of considerable interest in industry as well as in academia because of their promising biological activities as calcium channel blockers, antihypertensive agents, and anticancer drugs. [298, 299] They also show anti fungal activity, [300] antibacterial activity, [300] antiviral activity [301] and antitumor [301] activity. They are also known as analgesics [302] and antidepressants. [303] Thus, synthesis of this heterocyclic nucleus is of much importance, and quite a number of synthetic procedures based on the modifications of the century-old Biginelli's reaction involving acid-catalyzed three-component condensation of 1,3-dicarbonyl compound, aldehyde, and urea, have been developed during past few years. [298, 299] Basically, these methods are all similar, using different Lewis acid catalysts such as BF_3, [304a] $FeCl_3$, [304b] $InCl_3$, [304c] $BiCl_3$, [304d] $LaCl_3$, [304e] $LiClO_4$, [304f] Mn-$(OAc)_3$, [304g] CAN, [304h] and VCl_3 [304i] in a solvent such as CH_3CN, CH_2Cl_2, or THF. Recently, a number of procedures under solvent-free conditions using $Yb(OTf)_3$, [304j] montmorillonite [304k] and ionic liquid [304l] as catalysts have also been reported.

Biginelli reaction consists three components i.e. aldehyde functional group, urea or thiourea and β-keto ester. Many reactions have been reported using differently substituted aromatic as well as aliphatic aldehydes. Substituted ureas and thioureas have also been used in the synthesis of *N*-substituted dihydropyrimidine derivatives as well. Simple β-keto esters *viz.* ethylacetoacetate, methylacetoacetate and acetylacetone have been widely

used while differently substituted β-keto esters have also been used for the synthesis of diversified dihydropyrimidine derivatives via Biginelli reaction.

Use of 4-hydroxycoumarin in the synthesis of dihydropyrimidine synthesis is not much reported. The tautomeric form (1) of 4-hydroxy-coumarin acts as a cyclic β-keto ester (Fig. 4.27) and condenses with aldehydes in the presence of urea / thiourea under acidic conditions and gives rise to the expected coumarin fused pyrimidines.

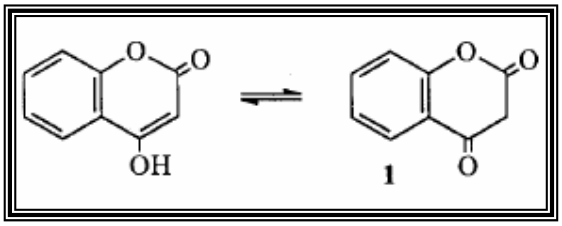


Fig. 4.27

Brambhatt *et. al.* [305] reported synthetic method for the fused benzopyranopyrimidine derivatives. They reported the poor yielded synthetic procedure with the longer time period.

Microwave assisted green chemical approach was applied to the synthesis of benzopyranopyrimidines by Kidwai *et. al.* [306] This method not only gave better yield with reduced reaction time but also eliminated the need of an external acid due to the usage of acidic solid support. Since the reaction takes place under acidic conditions, the effect of three different acidic inorganic support *viz.* acidic alumina, montmorillonite K10 clay and silica gel was explored. The same reaction was also carried out in solution phase under MW. A conventional method with basic alumina in oil bath maintained at 110–120°C was also attempted, but the reaction took from 4 to 6 h to give the required product in about 39–60 % yield. This clearly indicates that the coupling of microwaves with the solid supported reagent is more than simple thermal effects. Furthermore They [307] eliminated the use of inorganic solid support and condensed all the three components i.e. 4-hydroxycoumarin, aromatic aldehydes and urea / thiourea, neat under microwave irradiation.

4.6 AIM OF CURRENT WORK

This chapter contains four different schemes. Different types of reactions have been carried out on pyrazole aldehydes in each and every scheme. The synthesis of pyrazoles remain of great interest owing to the wide applications in pharmaceutical and agrochemical industry due to their herbicidal, fungicidal, insecticidal, analgesic, antipyretic, anti inflammatory, anti bacterial, anti parasitic and anti diabetic properties. Earlier, from this laboratory, some indolinone derivatives were prepared and tested for anti cancer activity on colon cancer cell line (SW 620), which showed good results. [c] In continuation of our previous work, few differently substituted pyrazole aldehydes were developed and new indolinone derivatives to observe their anti cancer activity.

Similarly, chalcones of 3-acetyl-4-hydroxycoumarin with differently substituted benzaldehydes were prepared and tested for anti viral activity which showed good results. In continuation, new chalcones of 3-acetyl-4-hydroxycoumarin with pyrazole aldehydes were synthesized and evaluated for their antiviral activity.

Recently much attention has been devoted towards dihydropyrimidine derivatives due to their significant therapeutic and medicinal properties. Literature survey revealed that differently substituted benzaldehydes are fused with 4-hydroxycoumarin and urea / thiourea to synthesize coumarin fused pyrimidines but in place of substituted benzaldehydes, pyrazole aldehydes are not approached, which inspired us to synthesize some new coumarin fused pyrimidines. In the present work, the keto tautomeric form of 4-hydroxy-coumarin acts as a cyclic β-keto ester and condenses with pyrazole aldehydes in the presence of urea / thiourea under acidic conditions and gives rise to the expected coumarin fused pyrimidines.

[c] V. Virsodia, A. Manvar, K. Upadhyay, R. Loriya, D. Karia, M. Jaggi, A. Singh, R. Mukherjee, M. S. Shaikh, E. C. Coutinho and A. Shah; *Eur. J. Med. Chem.*, **2008**, 1-8 (In press)

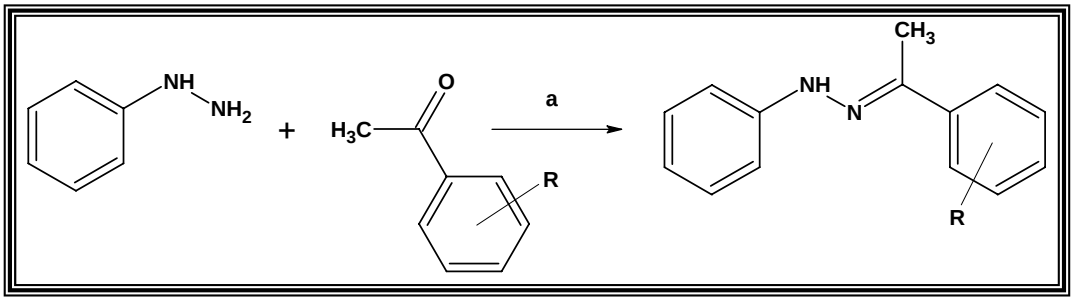
4-hydroxycoumarins and aromatic aldehydes are known to produce 2, 4-chromane diones (arylidine at C_3 position) and coumarin dimers under reflux, with or without base. Thus, few new chromane diones were prepared using pyrazole aldehydes.

Though the chemistry of the synthesized compounds is known, the compounds are reported herein for the first time. Biological importance of such important compounds is the rational behind the current work done in this chapter.

4.7 REACTION SCHEMES

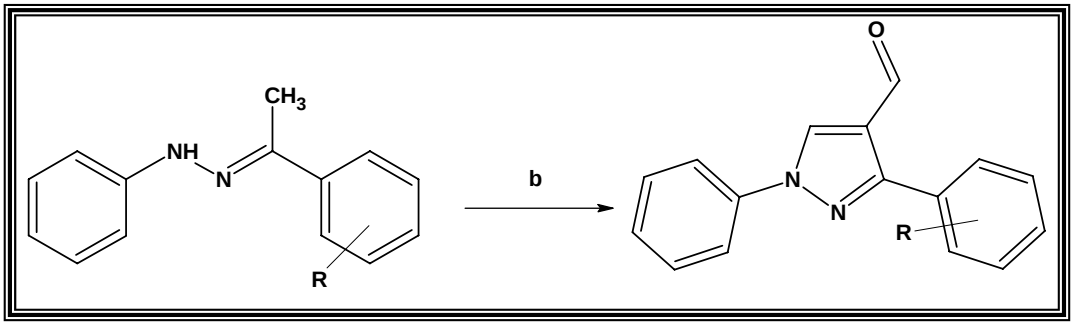
4.7.1 PREPARATION OF PYRAZOLE ALDEHYDE

STEP – 1



Reagents / Reaction Condition (a): Phenyl hydrazine, Substituted acetophenone, Glacial acetic acid, Ethanol / Reflux, 5-6 hours.

STEP – 2

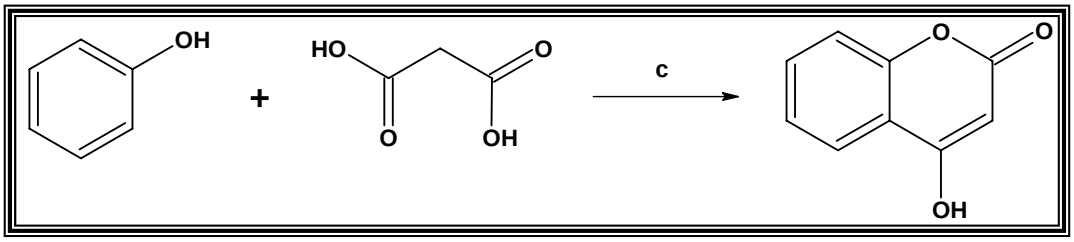


Reagents / Reaction Condition (b): DMF – $POCl_3$ / 70-80°C, 5-6 hours.

Where, R = 4-H, 4-Cl, 4-F, 4-NO_2, 3-NO_2

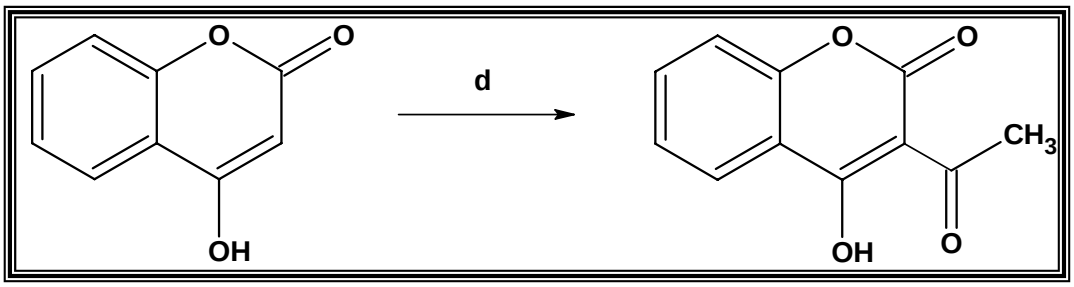
4.7.2 PREPARATION OF 3-ACETYL-4-HYDROXYCOUMARIN

STEP – 1



Reagents / Reaction Condition (c): Anhydrous $ZnCl_2$, $POCl_3$ / 70°C, 12 hours.

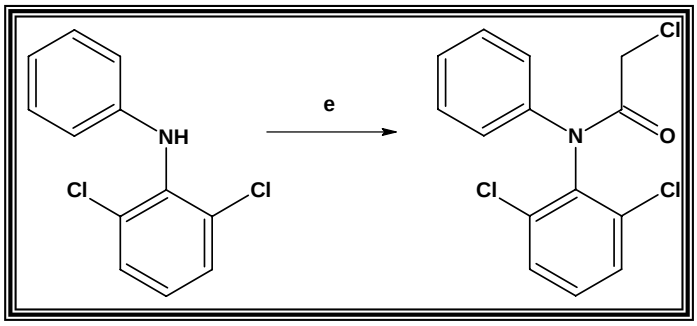
STEP – 2



Reagents / Reaction Condition (d): $POCl_3$, Glacial acetic acid / Reflux, 30 min.

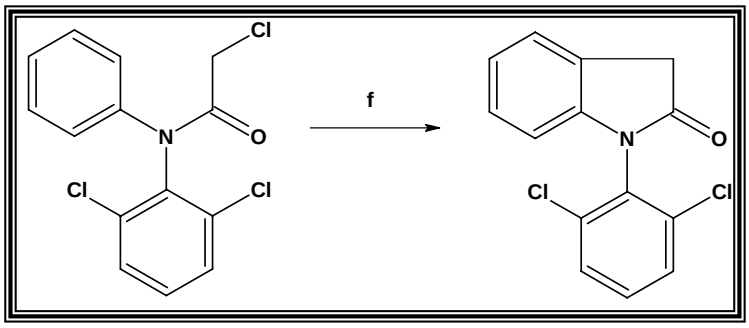
4.7.3 PREPARATION OF 1-(2, 6-DICHLOROPHENYL)-2-INDOLINONE

STEP – 1



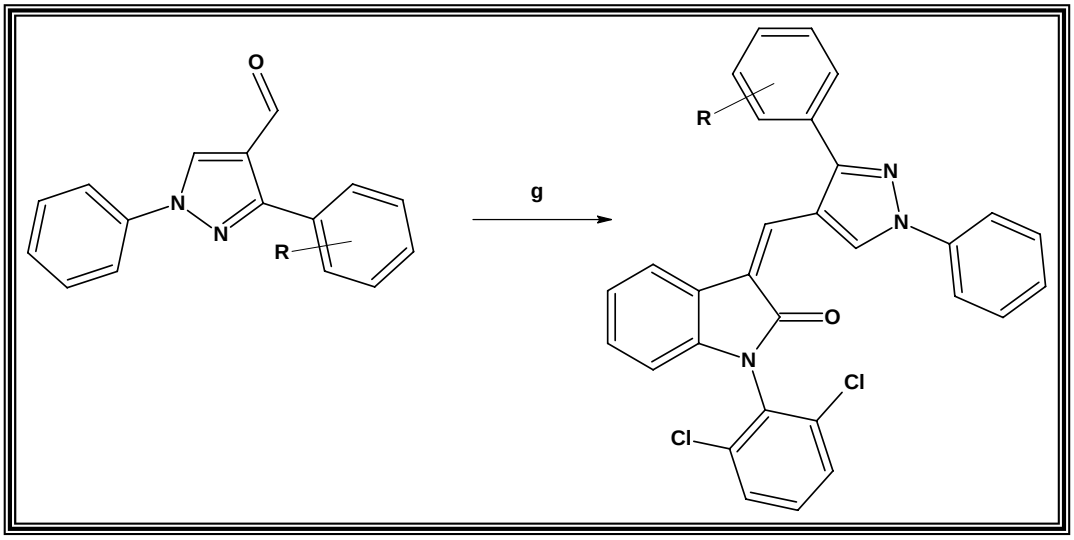
Reagents / Reaction Condition (e): $ClCH_2COCl$ / Reflux, 5 hours.

STEP – 2



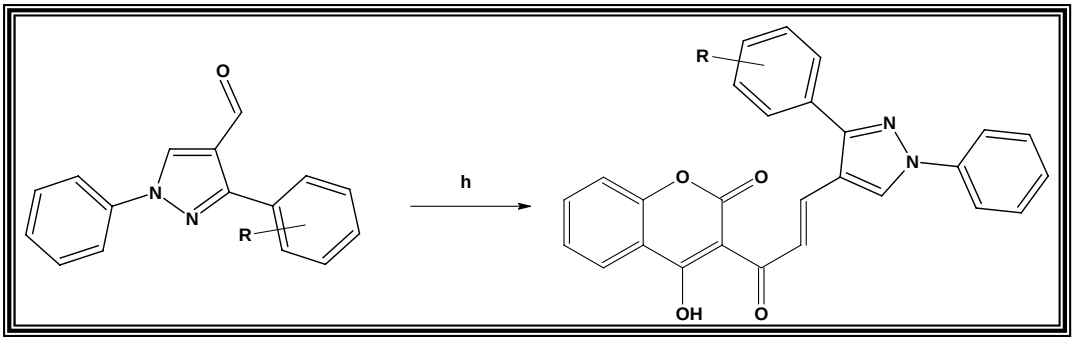
Reagents / Reaction Condition (f): Anhydrous $AlCl_3$ / 160°C, 2 hours.

4.7.4 SCHEME - 1



Reagents / Reaction Condition (g): 1-(2, 6-dichloro phenyl)-indolinone, Piperidine, Ethanol / Reflux, 5-7 hours. **OR** MW method: 1-(2, 6-dichloro phenyl)-indolinone, Piperidine, DMF / MW, 400 Watt.

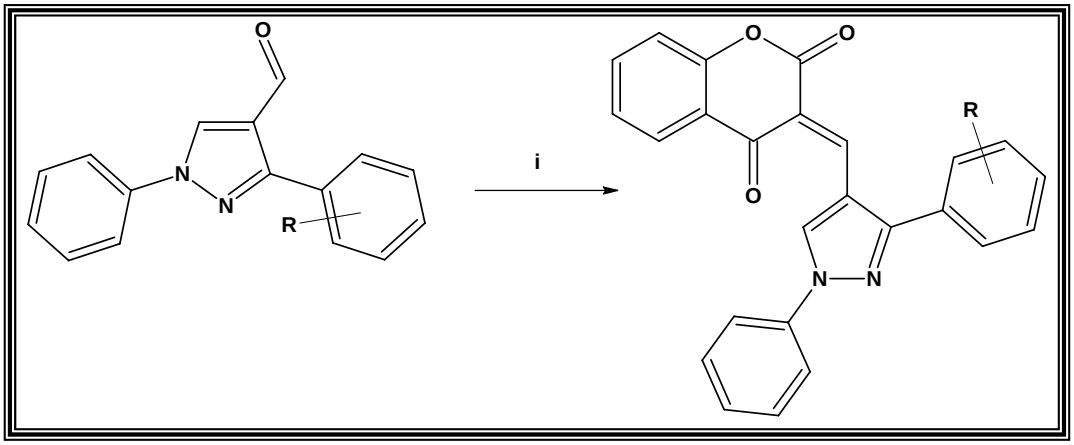
4.7.5 SCHEME - 2



Reagents / Reaction Condition (h): 3-acetyl-4-hydroxycoumarin, Piperidine, Chloroform / Reflux, 4 hours.

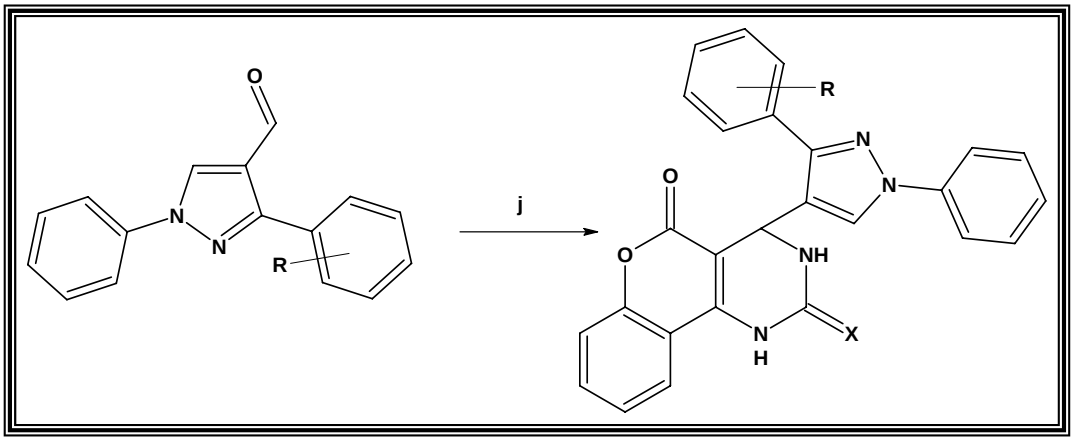
Where, R = 4-H, 4-Cl, 4-F, 4-NO_2, 3-NO_2

4.7.6 SCHEME - 3



Reagents / Reaction Condition (i): 4-hydroxycoumarin, Piperidine, Methanol / Reflux, 4-5 hours. **OR** MW method: 4-hydroxycoumarin, Piperidine, DMF / MW, 400 Watt.

4.7.7 SCHEME - 4

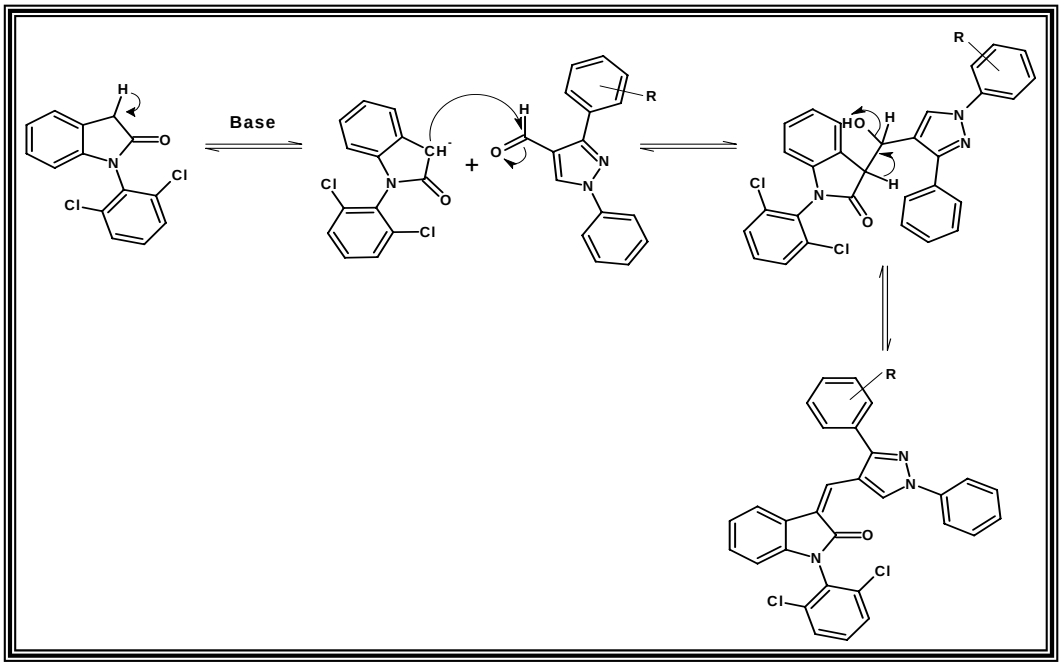


Reagents / Reaction Condition (j): 4-hydroxycoumarin, Urea or Thiourea, Con. HCl, Methanol / Reflux, 5-6 hours.

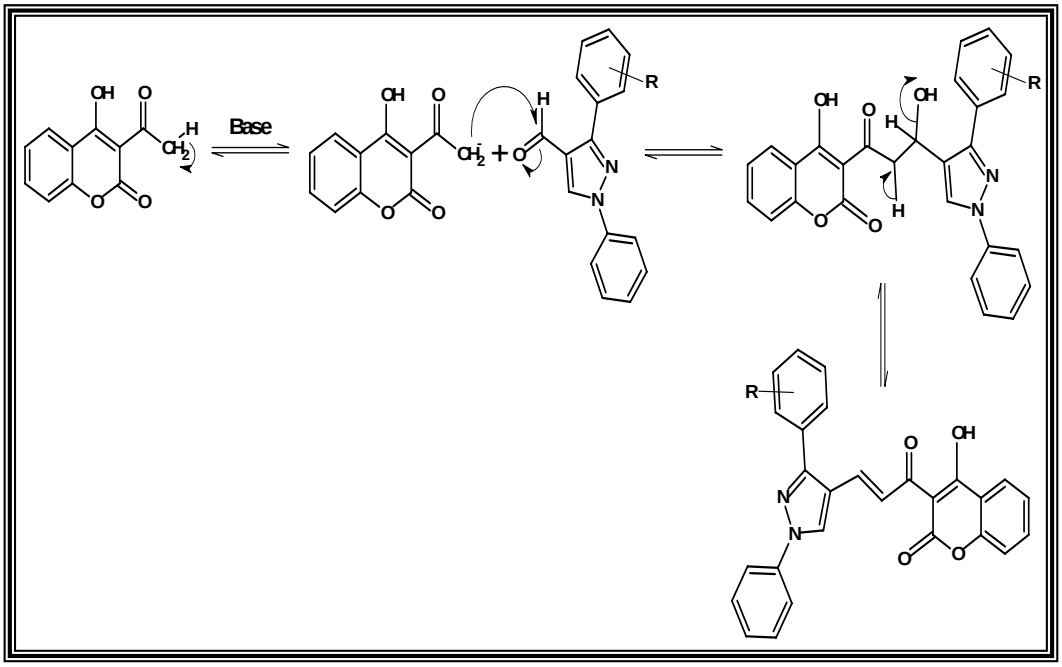
Where, X = O or S, R = 4-H, 4-Cl, 4-F, 4-NO_2, 3-NO_2

4.8 PLAUSIBLE REACTION MECHANISM

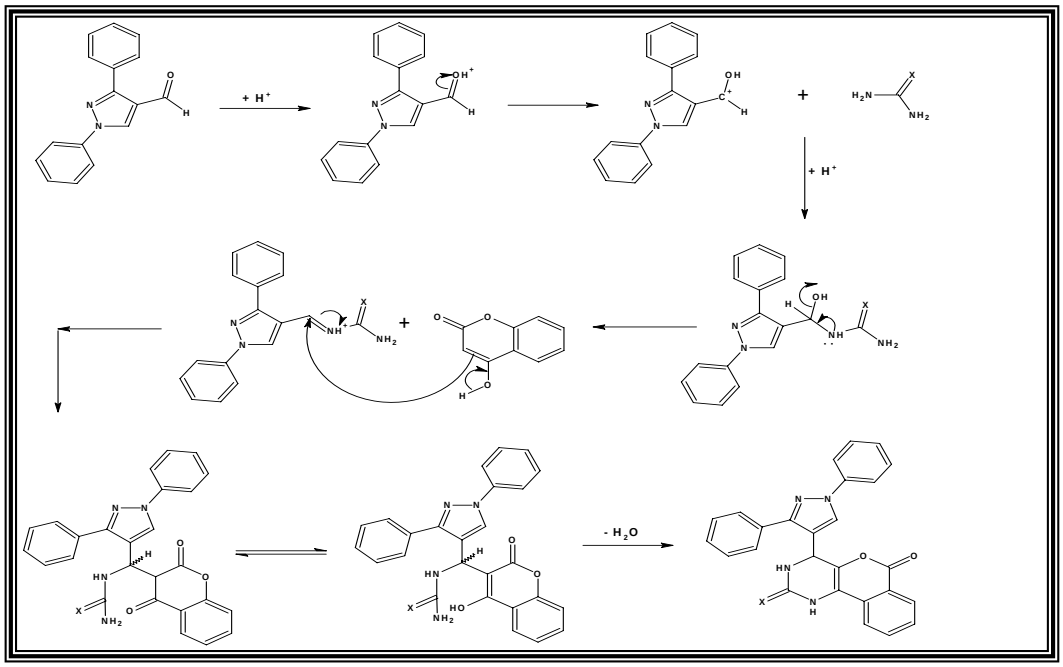
4.8.1 SCHEME - 1



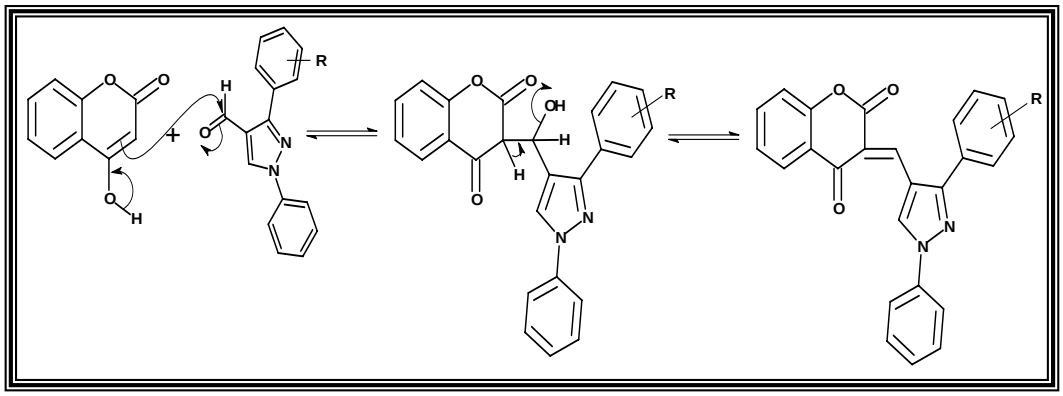
4.8.2 SCHEME - 2



4.8.3 SCHEME - 3



4.8.4 SCHEME - 4



4.9 EXPERIMENTAL

4.9.1 PREPARATION OF PYRAZOLE ALDEHYDE

STEP – 1 PREPARATION OF ACETOPHENONE PHENYL HYDRAZONE

0.1 mole of appropriately substituted acetophenone was dissolved in 50 ml of ethanol into 250 ml round bottom flask. 0.1 mole of Phenyl hydrazine was added to above flask along with 3-4 drops of glacial acetic acid. The reaction mixture was refluxed for 5-6 hours. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using toluene : ethyl acetate (7 : 3) as a mobile phase. After the reaction to be completed, the reaction mixture was cooled to room temperature and the crystalline product was separated by filtration. The product was washed with ethanol and dried to give substituted acetone phenyl hydrazone in good yield which was pure enough to use as such for the next step.

Code No.	MF	MW (g/m)	MP (°C)	% Yield
APH-01	$C_{14}H_{13}N_3O_2$	255	106-108	85
APH-02	$C_{14}H_{13}N_3O_2$	255	134-136	80
APH-03	$C_{14}H_{14}N_2$	210	126-128	78
APH-04	$C_{14}H_{13}ClN_2$	244	-----	79
APH-05	$C_{14}H_{13}FN_2$	228	-----	72

STEP – 2 PREPARATION OF PYRAZOLE ALDEHYDE

25 ml of dry dimethylformamide was transferred into 250 ml flat bottom flask. 3 ml of phosphorous oxychloride was added drop wise to above flask under stirring at 0-5°C. After completion of the addition, the mixture was stirred at this temperature for 10-15 min. 0.03 mole of freshly prepared acetophenone phenyl hydrazone was added to above mixture and the content was heated on water bath for 5-6 hours. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using toluene : ethyl acetate (7 : 3) as a mobile phase. After the reaction to be completed, the reaction mixture was cooled to room temperature and the

content of the flask was poured on crushed ice to isolate the product. The separated product was filtered off and it was washed with cold water to remove acidity. It was dried at 65°C and recrystallized from the mixture of DMF-Methanol to give crystalline pyrazole aldehyde in good yield.

Code No.	MF	MW (g/m)	MP (°C)	% Yield
PA-01	$C_{16}H_{11}N_3O_3$	293	162-164	75
PA-02	$C_{16}H_{11}N_3O_3$	293	176-178	70
PA-03	$C_{16}H_{12}N_2O$	248	144-146	68
PA-04	$C_{16}H_{11}ClN_2O$	282	142-144	71
PA-05	$C_{16}H_{11}FN_2O$	266	148-150	70

4.9.2 PREPARATION OF 3-ACETYL-4-HYDROXYCOUMARIN

STEP – 1 PREPARATION OF 4-HYDROXYCOUMARIN

It was prepared according to the method reported by Shah *et. al.* [d] Yield - 55 %, MP - 210-212°C. (210-212°C [b])

STEP – 2 PREPARATION OF 3-ACETYL-4-HYDROXYCOUMARIN

It was prepared according to the method reported by Dholakia *et. al.* [e] Yield - 60 %, MP - 120-122°C. (121-122°C [c])

4.9.3 PREPARATION OF 1-(2, 6-DICHLOROPHENYL)-2-INDOLINONE

It was prepared according to the literature method. [f, g, h, i] Yield - 60 %, MP - 126-128°C. (124°C-126°C [e])

[d] A. K. Shah, N. S. Bhatt and V. M. Thakor; *Curr. Sci.*, **1984**, *53(24)*, 1289.

[e] V N. Dholakia, M. G. Parekh and K. N. Trivedi; *Aust. J. Chem.*, **1968**, *21*, 2345.

[f] G. S. Predvoditeleva, T. V. Kartseva, O. N. Oleshko, V. I. Shvedov, R. D. Syubaev, G. Ya. Shvarts, L. M. Alekseeva, O. S. Shvedov, V. V. Chistyakov and Yu. N. Sheinker; *Khim.-Farm. Zh.*, **1987**, *21*, 441.

[g] P. Moser, A. Sallmann and I. Wiesenberg; *J. Med. Chem.*, **1990**, *33*, 2358.

[h] A. Sallmann and R. Pfister; *British Patatent 1,132,318*, **1968**. *(CA 70:57455b)*

[i] A. Sallmann and R. Pfister; *Ger. Offen. 1,815,802*, **1969**. *(CA 72:12385d)*

4.9.4 GENERAL PROCEDURE FOR THE PREPARATION OF DNJ-701 TO DNJ-705

METHOD – (A) CONVENTIONAL APPROACH

0.01 mole of 1-(2, 6-dichlorophenyl)-2-indolinone was dissolved into 30 ml of methanol into 100 ml round bottom flask. 0.01 mole of an appropriately substituted pyrazole-4-carboxaldehyde was added to the above flask along with catalytic amount of piperidine. The reaction mixture was refluxed on water bath for 5-7 hours. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using hexane : ethyl acetate (6 : 4) as a mobile phase. After the reaction to be completed, the reaction mixture was cooled to room temperature and the product was separated by filtration. The product was washed with methanol and dried to give desired product in moderate yield which was recrystallized by DMF. (Physical data of the synthesized end products are summarized in the table 4.10.1)

METHOD – (B) MICROWAVE APPROACH

0.01 mole of 1-(2, 6-dichlorophenyl)-2-indolinone was dissolved into 20 ml of dimethylformamide into 100 ml microwave flask. 0.01 mole of an appropriately substituted pyrazole-4-carboxaldehyde was added to the above flask along with catalytic amount of piperidine. The reaction mixture was irradiated under microwave irradiation using Qpro-M microwave synthesizer for the desired time at 400 W. The progress and the completion of the reaction were checked at the interval of every one min. by silica gel-G F_{254} thin layer chromatography using hexane : ethyl acetate (6 : 4) as a mobile phase. After the reaction to be completed, the reaction mixture was cooled and scratched into 30 ml of methanol. The separated product was filtered off and washed with methanol and it was dried to give desired product in good yield which was recrystallized by DMF. (Physical data of the synthesized end products are summarized in the table 4.10.1)

Comparative results of method (A) and method (B) are summarized as under.

Code No.	Reaction Condition					% Yield	
	Method (A)		Method (B)			Method (A)	Method (B)
	Temp. (°C)	Time (hrs.)	Watt (W)	Temp. (°C)	Time (min.)		
DNJ-701	90-95	5.0	400	110	3.0	48	81
DNJ-702	90-95	5.5	400	110	3.1	50	80
DNJ-703	90-95	6.0	400	110	3.3	52	85
DNJ-704	90-95	7.0	400	110	3.0	49	79
DNJ-705	90-95	4.0	400	110	3.2	55	83

4.9.5 GENERAL PROCEDURE FOR THE PREPARATION OF DNJ-801 TO DNJ-805

0.01 mole of 3-acetyl-4-hydroxycoumarin and 0.01 mole of an appropriately substituted pyrazole-4-carboxaldehyde were dissolved in 30 ml of chloroform. A catalytic amount of piperidine was added and the reaction mixture was refluxed for 4 hours. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using hexane : ethyl acetate (6 : 4) as a mobile phase. The chloroform was distilled out and the residue was washed with methanol and dried to give desired product in moderate yield which was recrystallized by DMF. (Physical data of the synthesized end products are summarized in the table 4.10.2)

4.9.6 GENERAL PROCEDURE FOR THE PREPARATION OF DNJ-901 TO DNJ-905

METHOD – (A) CONVENTIONAL APPROACH

0.01 mole of 4-hydroxycoumarin was dissolved in 30 ml of methanol into 100 ml round bottom flask. 0.01 mole of an appropriately substituted pyrazole-4-carboxaldehyde was added to the above flask along with few drops of piperidine. The reaction mixture was heated on water bath for 4-5 hours. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using hexane : ethyl acetate (6 : 4) as a

mobile phase. After the reaction to be completed, the reaction mixture was cooled to room temperature and the product was separated by filtration. The product was washed with methanol and dried to give desired product in good yield which was recrystallized by DMF. (Physical data of the synthesized end products are summarized in the table 4.10.3)

METHOD – (B) MICROWAVE APPROACH

0.01 mole of 4-hydroxycoumarin was dissolved into 20 ml of dimethylformamide into 100 ml microwave flask. 0.01 mole of an appropriately substituted pyrazole-4-carboxaldehyde was added to the above flask along with few drops of piperidine. The reaction mixture was irradiated under microwave irradiation using Qpro-M microwave synthesizer for the desired time at 400 W. The progress and the completion of the reaction were checked at interval of every one min. by silica gel-G F_{254} thin layer chromatography using hexane : ethyl acetate (6 : 4) as a mobile phase. After the reaction to be completed, the reaction mixture was scratched into 30 ml of methanol. The separated product was filtered off and washed with methanol and it was dried to give desired product in good yield which was recrystallized by DMF. (Physical data of the synthesized end products are summarized in the table 4.10.3)

Comparative results of method (A) and method (B) are summarized as under.

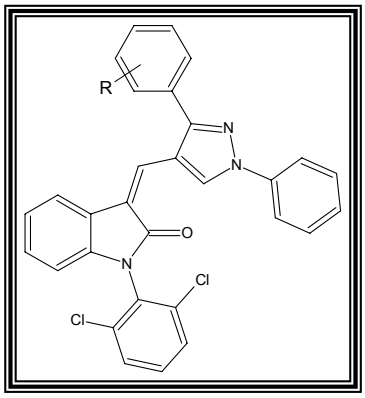
Code No.	Reaction Condition					% Yield	
	Method (A)		Method (B)			Method (A)	Method (B)
	Temp. (°C)	Time (hrs.)	Watt (W)	Temp. (°C)	Time (min.)		
DNJ-901	90-95	4.5	400	110	3.0	68	85
DNJ-902	90-95	5.0	400	110	3.3	64	80
DNJ-903	90-95	4.8	400	110	3.1	70	83
DNJ-904	90-95	5.5	400	110	3.2	72	81
DNJ-905	90-95	5.3	400	110	3.0	66	79

4.9.7 GENERAL PROCEDURE FOR THE PREPARATION OF DNJ-1601 TO DNJ-1605 AND DNJ-1701 TO DNJ-1705

0.015 mole of urea / thiourea was dissolved into 20 ml of methanol. 0.01 mole of an appropriately substituted pyrazole-4-carboxaldehyde was added to above reaction mixture and additional 20 ml of methanol was added along with few drops of concentrated hydrochloric acid. This reaction mixture was transferred to 250 ml round bottom flask containing 0.01 mole of 4-hydroxycoumarin into 10 ml of methanol. The content of the flask was heated on water bath for 5-6 hours. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using toluene : ethyl acetate (7 : 3) as a mobile phase. After the reaction to be completed, the reaction mixture was cooled to room temperature and the product was separated by filtration. The product was washed with methanol and dried to give desired product in moderate yield which was recrystallized by DMF. (Physical data of the synthesized end products are summarized in the table 4.10.4 & 4.10.5)

4.10 PHYSICAL DATA TABLES

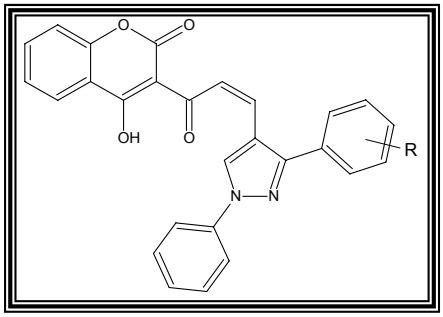
4.10.1 Physical data of 1-(2, 6-dichlorophenyl)-3-{[3-(substituted phenyl)-1-phenyl-1*H*-pyrazol-4-yl] methylene}-1, 3-dihydro-2*H*-indol-2-one (DNJ-701 to DNJ-705)



Code	Substitution R	MF	MW (g/m)	MP (°C)	R_f
DNJ-701	4-NO_2	$C_{30}H_{18}Cl_2N_4O_3$	553	250-252	0.62
DNJ-702	3-NO_2	$C_{30}H_{18}Cl_2N_4O_3$	553	268-270	0.61
DNJ-703	H	$C_{30}H_{19}Cl_2N_3O$	508	224-226	0.55
DNJ-704	4-F	$C_{30}H_{18}Cl_2FN_3O$	526	240-242	0.57
DNJ-705	4-Cl	$C_{30}H_{18}Cl_3N_3O$	542	256-258	0.60

R_f value was calculated using solvent system = Hexane : Ethyl Acetate (6 : 4)

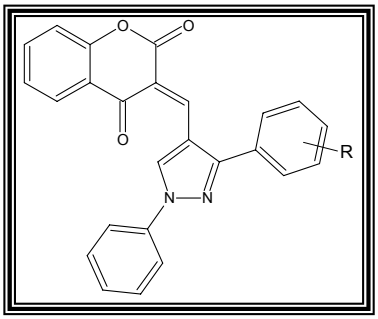
4.10.2 Physical Data of 3-{(2*E*)-3-[3-(substituted phenyl)-1-phenyl-1*H*-pyrazol-4-yl] prop-2-enoyl}-4-hydroxy-2*H*-chromen-2-one (DNJ-801 to DNJ-805)



Code	Substitution R	MF	MW (g/m)	MP (°C)	R_f	% Yield
DNJ-801	H	$C_{27}H_{18}N_2O_4$	434	270-272	0.46	50
DNJ-802	4-NO_2	$C_{27}H_{17}N_3O_6$	479	262-264	0.52	52
DNJ-803	3-NO_2	$C_{27}H_{17}N_3O_6$	479	222-224	0.50	48
DNJ-804	4-Cl	$C_{27}H_{17}ClN_2O_4$	468	250-252	0.44	54
DNJ-805	4-F	$C_{27}H_{17}FN_2O_4$	452	264-266	0.49	51

R_f value was calculated using solvent system = Hexane : Ethyl Acetate (6 : 4)

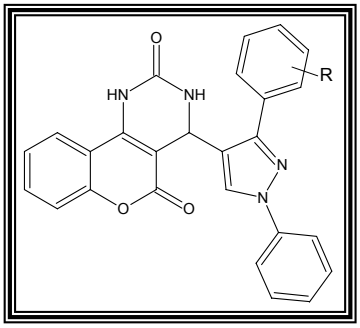
4.10.3 Physical data of (3*E*)-3-{[3-(substituted phenyl)-1-phenyl-1*H*-pyrazol-4-yl] methylene}-2*H*-chromene-2, 4(3*H*)-dione (DNJ-901 to DNJ-905)



Code	Substitution R	MF	MW (g/m)	MP (°C)	R_f
DNJ-901	4-NO_2	$C_{25}H_{15}N_3O_5$	437	162-164	0.45
DNJ-902	3-NO_2	$C_{25}H_{15}N_3O_5$	437	170-172	0.47
DNJ-903	H	$C_{25}H_{16}N_2O_3$	392	158-160	0.40
DNJ-904	4-Cl	$C_{25}H_{15}ClN_2O_3$	426	178-180	0.50
DNJ-905	4-F	$C_{25}H_{15}FN_2O_3$	410	184-186	0.42

R_f value was calculated using solvent system = Hexane : Ethyl Acetate (6 : 4)

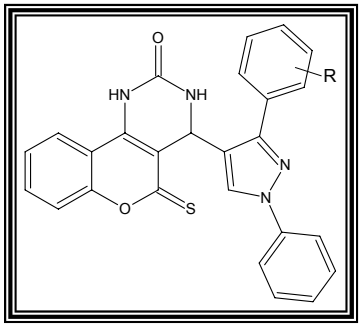
4.10.4 Physical data of 4-[3-(substituted phenyl)-1-phenyl-1*H*-pyrazol-4-yl]-3, 4-dihydro-2*H*-chromeno [4, 3-d] pyrimidine-2, 5(1*H*)-dione (DNJ-1601 to DNJ-1605)



Code	Substitution R	MF	MW (g/m)	MP (°C)	R_f	% Yield
DNJ 1601	H	$C_{26}H_{18}N_4O_3$	434	278-280	0.50	48
DNJ 1602	4-NO_2	$C_{26}H_{17}N_5O_5$	479	288-290 (dec.)	0.53	51
DNJ 1603	3-NO_2	$C_{26}H_{17}N_5O_5$	479	294-296 (dec.)	0.51	54
DNJ 1604	4-Cl	$C_{26}H_{17}ClN_4O_3$	468	286-288	0.48	44
DNJ 1605	4-F	$C_{26}H_{17}FN_4O_3$	452	282-284	0.46	56

R_f value was calculated using Solvent System = Toluene : Ethyl Acetate (7 : 3)

4.10.5 Physical data of 4-[3-(substituted phenyl)-1-phenyl-1*H*-pyrazol-4-yl]-5-thioxo-1, 3, 4, 5-tetrahydro-2*H*-chromeno [4, 3-d] pyrimidin-2-one (DNJ-1701 to DNJ-1705)



Code	Substitution R	MF	MW (g/m)	MP (°C)	R_f	% Yield
DNJ 1701	H	$C_{26}H_{18}N_4O_2S$	450	288-290	0.51	50
DNJ 1702	4-NO_2	$C_{26}H_{17}N_5O_4S$	495	298-300 (dec.)	0.59	54
DNJ 1703	3-NO_2	$C_{26}H_{17}N_5O_4S$	495	>300	0.57	48
DNJ 1704	4-Cl	$C_{26}H_{17}ClN_4O_2S$	484	292-294	0.55	48
DNJ 1705	4-F	$C_{26}H_{17}FN_4O_2S$	468	296-298	0.52	52

R_f value was calculated using Solvent System = Toluene : Ethyl Acetate (7 : 3)

4.11 SPECTRAL DISCUSSION

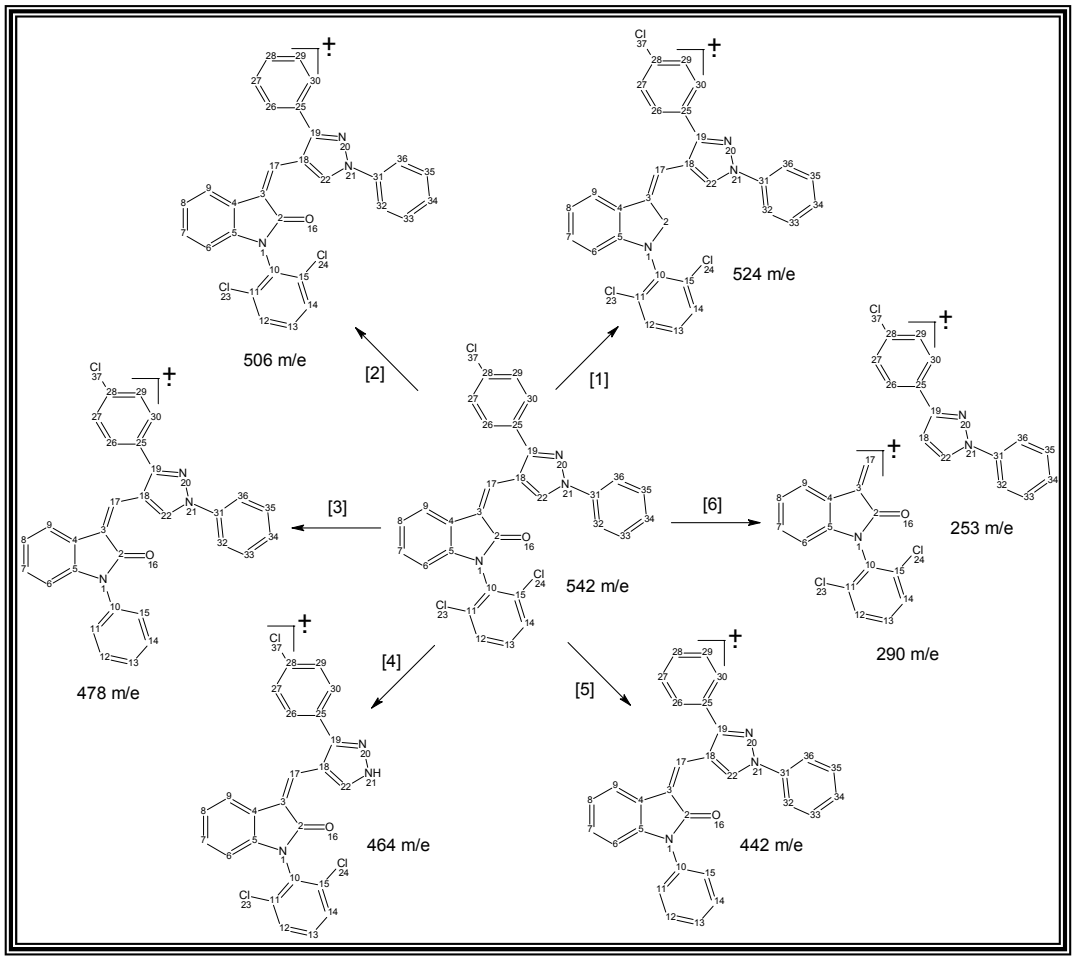
4.11.1 MASS SPECTRAL STUDY

Mass spectra of the synthesized compounds were recorded on **Shimadzu GC-MS QP-2010** model using direct injection probe technique. The molecular ion peak was found in agreement with molecular weight of the respective compound. Characteristic M^{+2} ion peaks with one-third intensity of molecular ion peak were observed in case of compounds having chlorine atom. Fragmentation pattern can be observed to be particular for these compounds and the characteristic peaks obtained for each compound. Probable fragmentation pattern for DNJ-705, DNJ-804, DNJ-905, DNJ-1605 and DNJ-1705 can be discussed as under.

3-{[3-(4-chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl] methylene}-1-(2, 6-dichlorophenyl)-1, 3-dihydro-2*H*-indol-2-one (DNJ-705)

1. The target compound showed characteristic molecular ion peak.
2. C_2-C_{16} bond cleavage gave characteristic peak at 524 m/e. [1]
3. Loss of one chlorine atom (C_{28}-Cl_{37} bond cleavage) from title compound gave characteristic peak at 506 m/e. [2]
4. Loss of two chlorine atoms (C_{11}-Cl_{23} and C_{15}-Cl_{24} bond cleavages) gave characteristic peak at 478 m/e. [3]
5. N_{21}-C_{31} bond cleavage gave characteristic peak at 464 m/e. (loss of one phenyl ring) [4]
6. Loss of all the three chlorine atoms, from the title compound gave characteristic peak at 442 m/e. (C_{28}-Cl_{37}, C_{11}-Cl_{23} and C_{15}-Cl_{24} bond cleavages) [5]
7. C_{17}-C_{18} bond cleavage gave two characteristic peaks at 290 m/e and 253 m/e respectively. [6]

4.11.1.1 FRAGMENTATION PATTERN FOR DNJ-705

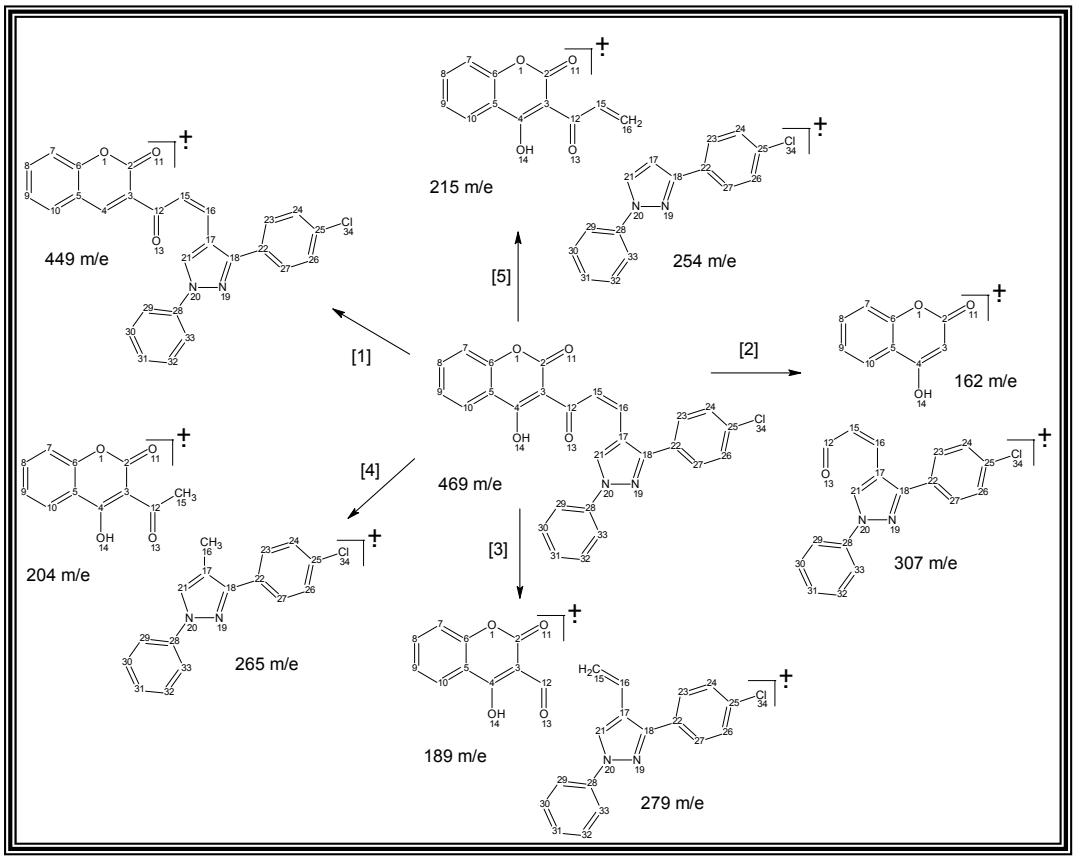


3-{(2*Z*)-3-[3-(4-chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl] prop-2-enoyl}-4-hydroxy-2*H*-chromen-2-one (DNJ-804)

1. The target compound showed characteristic molecular ion peak.
2. Loss of hydroxyl group, substituted at C_4 position, gave characteristic peak at 449 m/e. [1]
3. C_3-C_{12} bond cleavage gave two characteristic peaks at 307 m/e and 162 m/e respectively. [2]
4. C_{12}-C_{15} bond cleavage gave two characteristic peaks at 279 m/e and 189 m/e. [3]
5. C_{15}-C_{16} bond cleavage gave characteristic peak at 265 m/e. [4]

6. C_{16}-C_{17} bond cleavage gave characteristic peak at 215 m/e. [5]

4.11.1.2 FRAGMENTATION PATTERN FOR DNJ-804

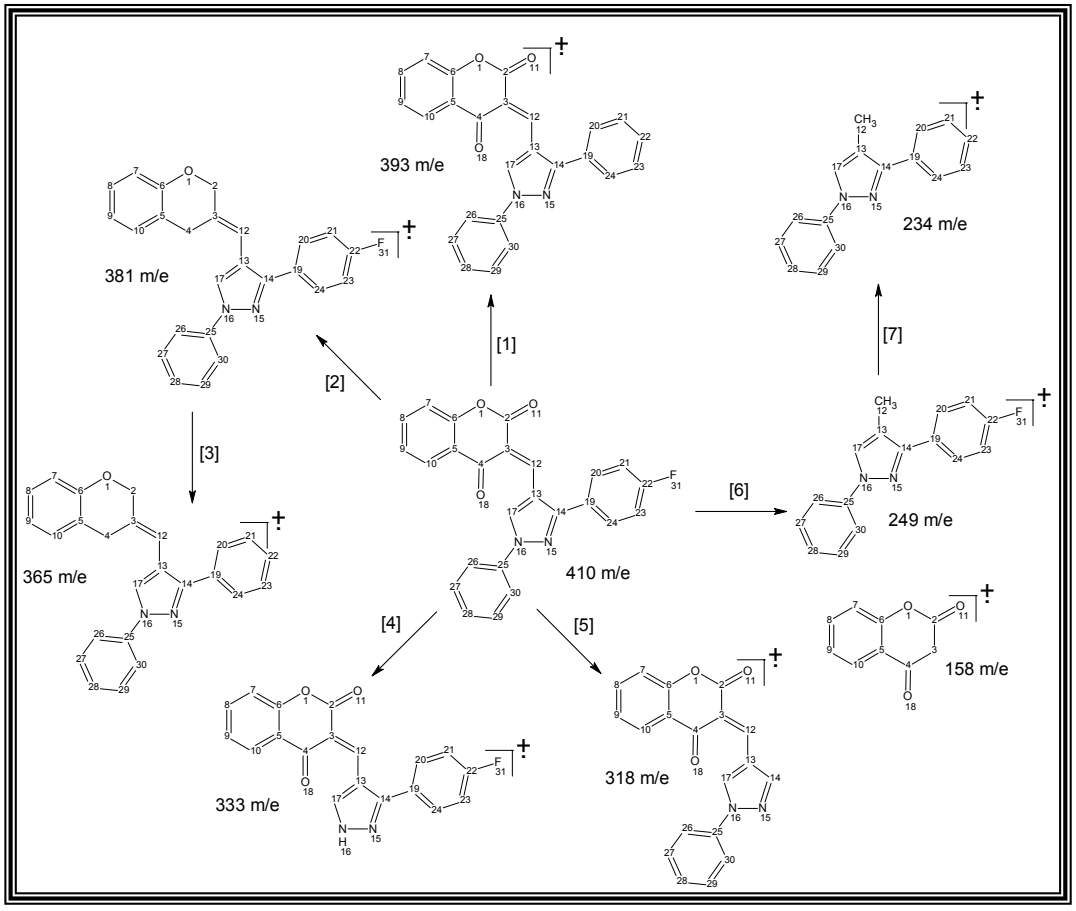


3-{[3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl] methylene}-2*H*-chromene-2, 4(3*H*)-dione (DNJ-905)

1. The target compound showed characteristic molecular ion peak (BASE PEAK.
2. Loss of fluorine atom gave characteristic peak at 393 m/e. [1]
3. Loss of two carbonyl groups, substituted at C_2 and C_4 position gave characteristic peak at 381 m/e (C_2-C_{11} and C_4-C_{18} bond cleavages). [2]
4. After C_2-C_{11} and C_4-C_{18} bond cleavages, loss of flurorine atom gave characteristic peak at 365 m/e (C_{22}-F_{31} bond cleavage). [3]
5. N_{16}-C_{25} bond cleavage gave characteristic peak at 333 m/e. [4]
6. C_{14}-C_{19} bond cleavage gave characteristic peak at 318 m/e. [5]

7. C_3-C_{12} bond cleavage gave two characteristic peaks at 249 m/e and 158 m/e respectively. [6]
8. After C_3-C_{12} bond cleavage, from 249 m/e fragment loss of fluorine atom gave characteristic peak at 234 m/e. [7]

4.11.1.3 FRAGMENTATION PATTERN FOR DNJ-905

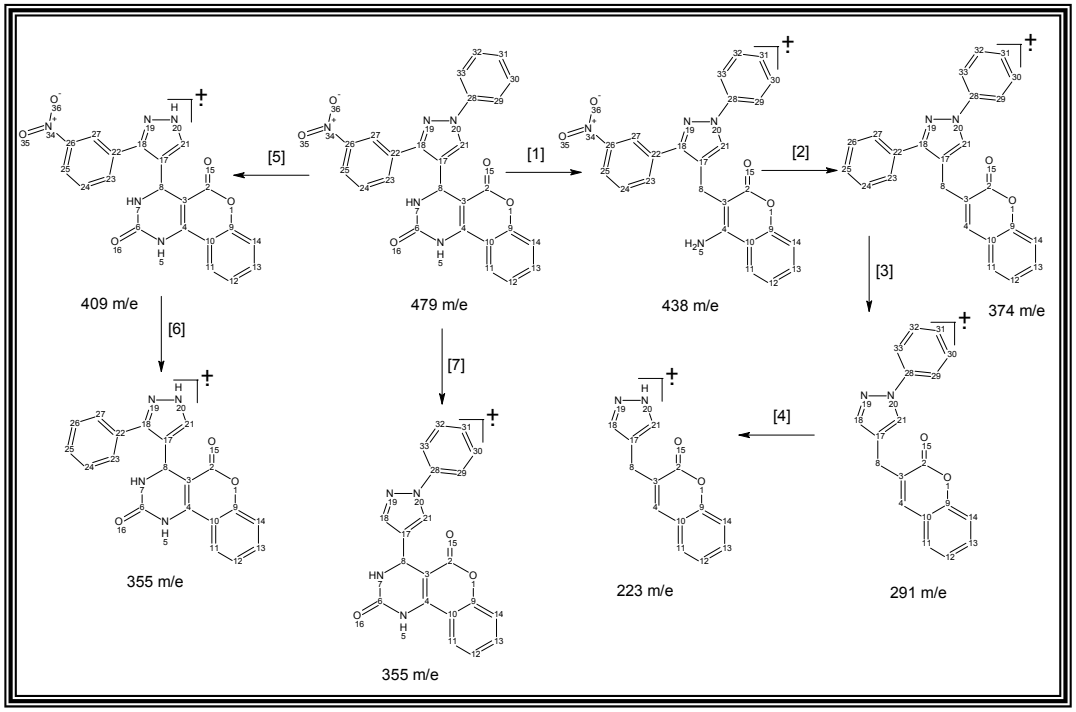


4-[3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl]-3, 4-dihydro-2*H*-chromeno [4, 3-*d*] pyrimidine-2, 5(1*H*)-dione (DNJ-1603)

1. The target compound showed characteristic molecular ion peak.
2. N_5-C_6 and N_7-C_8 bond cleavages gave characteristic peak at 438 m/e. [1]
3 After N_5-C_6 and N_7-C_8 bond cleavages, C_4-N_5 bond cleavage with subsequent loss of nitro group gave characteristic peak at 374 m/e. [2]

4. After C_4-N_5 bond cleavage and loss of nitro group, C_{18}-C_{22} bond cleavage gave characteristic peak at 291 m/e. [3]
5. After C_{18}-C_{22} bond cleavage, N_{20}-C_{28} bond cleavage gave characteristic peak at 223 m/e. [4]
6. From title molecule, N_{20}-C_{28} bond cleavage gave characteristic peak at 409 m/e. [5]
7. After N_{20}-C_{28} bond cleavage, loss of nitro group or C_{18}-C_{22} bond cleavage from title molecule gave characteristic peak at 355 m/e. [6 & 7]

4.11.1.4 FRAGMENTATION PATTERN FOR DNJ-1603



4-[3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl]-2-thioxo-1,2,3,4-tetrahydro-5*H*-chromeno [4, 3-*d*] pyrimidin-5-one (DNJ-1703)

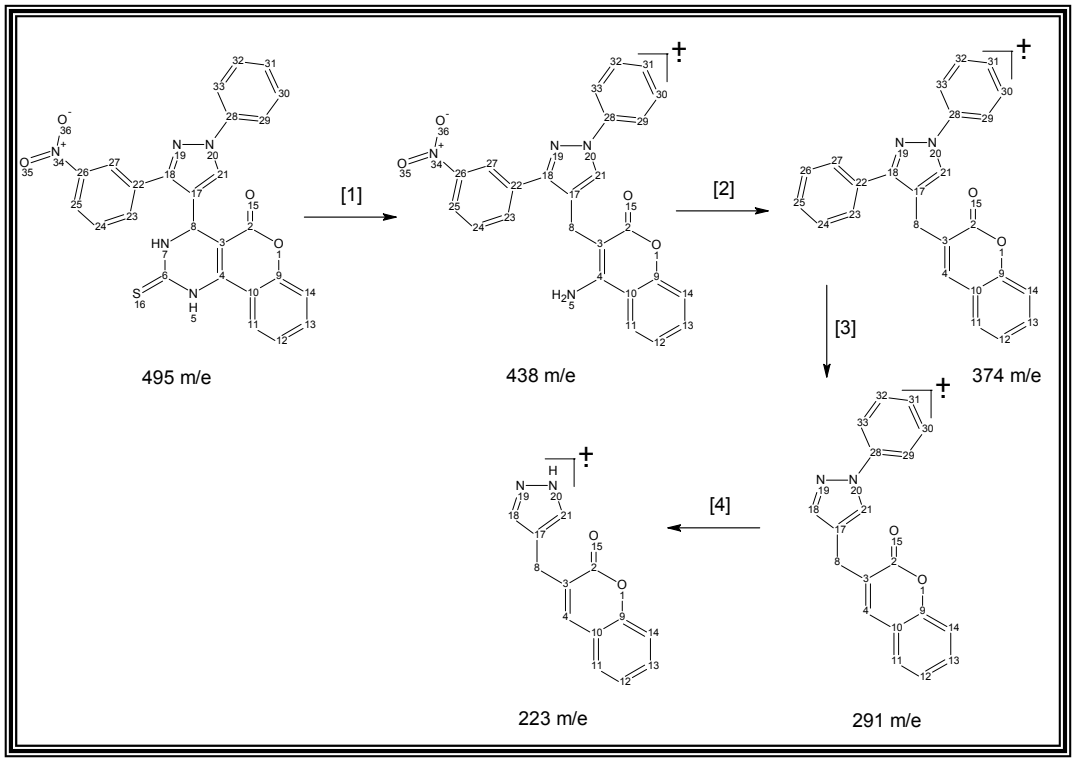
1. The target compound showed characteristic molecular ion peak.
2. N_5-C_6 and N_7-C_8 bond cleavages gave characteristic peak at 438 m/e. [1]

3 After N_5-C_6 and N_7-C_8 bond cleavages, C_4-N_5 bond cleavage with subsequent loss of nitro group gave characteristic peak at 374 m/e. [2]

4. After C_4-N_5 bond cleavage and loss of nitro group, C_{18}-C_{22} bond cleavage gave characteristic peak at 291 m/e. [3]

5. After C_{18}-C_{22} bond cleavage, N_{20}-C_{28} bond cleavage gave characteristic peak at 223 m/e. [4]

4.11.1.5 FRAGMENTATION PATTERN FOR DNJ-1703



4.11.2 IR SPECTRAL STUDY

IR spectra of the synthesized compounds were recorded on **Shimadzu FT IR 8400** spectrophotometer using Diffused Reflectance Attachment (DRA) System using Potassium Bromide.

In case of DNJ-701 to DNJ-705 series of compounds, stretching and bending frequency for aromatic and stretching frequencies for methyl,

methylene groups were found near 3050 cm^{-1}, 1400-1640 cm^{-1}, 2950 cm^{-1} and 2850 cm^{-1} respectively in all the compounds. Characteristic frequencies for carbonyl group (near 1700 cm^{-1}), C-Cl (near 870 cm^{-1}), C-N (3°) (near 1350 cm^{-1}) and 1, 2-disubstitution (near 750 cm^{-1}) in all the compounds. Characteristic frequency for 1, 4-disubstitution (near 820 cm^{-1}) was found in DNJ-701, DNJ-704 and DNJ-705 while for 1, 3-disubstitution (near 770 cm^{-1}) was found in DNJ-702. Characteristic frequencies for C-F (near 990 cm^{-1}), C-Cl (near 870 cm^{-1}) were found in DNJ-704 and DNJ-705 respectively.

In case of DNJ-801 to DNJ-805 series of compounds, stretching and bending frequency for aromatic and stretching frequencies for methyl, methylene groups were found near 3050 cm^{-1}, 1400-1640 cm^{-1}, 2950 cm^{-1} and 2850 cm^{-1} respectively in all the compounds. Characteristic frequency for hydroxyl group was obtained near 3600 cm^{-1} in all the compounds. Two Carbonyl stretching frequencies were obtained near 1700 cm^{-1} in all the compounds. Frequencies for ether linkage and C-N (3°) were obtained near 1050 cm^{-1} and 1350 cm^{-1} respectively in all the compounds. Characteristic frequency for 1, 4-disubstitution (near 820 cm^{-1}) was found in DNJ-802, DNJ-804 and DNJ-805 while for 1, 3-disubstitution (near 770 cm^{-1}) was found in DNJ-803. Characteristic frequencies for C-Cl (near 870 cm^{-1}), C-F (near 990 cm^{-1}) were found in DNJ-804 and DNJ-805 respectively.

In case of DNJ-901 to DNJ-905 series of compounds, stretching and bending frequency for aromatic and stretching frequencies for methyl, methylene groups were found near 3050 cm^{-1}, 1400-1640 cm^{-1}, 2950 cm^{-1} and 2850 cm^{-1} respectively in all the compounds. Two Carbonyl stretching frequencies were obtained near 1700 cm^{-1} and 1670 cm^{-1} in all the compounds. Frequencies for ether linkage and C-N (3°) were obtained near 1050 cm^{-1} and 1350 cm^{-1} respectively in all the compounds. Characteristic frequency for 1, 4-disubstitution (near 820 cm^{-1}) was found in DNJ-901, DNJ-904 and DNJ-905 while for 1, 3-disubstitution (near 770 cm^{-1}) was found in DNJ-802. Characteristic frequencies for C-Cl (near 870 cm^{-1}), C-F (near 990 cm^{-1}) were found in DNJ-904 and DNJ-905 respectively.

DNJ-1601 to DNJ-1605 and DNJ-1701 to DNJ-1705 serieses of compounds, almost similar kind of frequencies were observed. Stretching frequency for secondary amine group (-NH) was obtained near 3150 cm^{-1} in all the compounds. Stretching and bending frequency for aromatic and stretching frequencies for methyl, methylene groups were found near 3050 cm^{-1}, 1400-1640 cm^{-1}, 2950 cm^{-1} and 2850 cm^{-1} respectively in all the compounds. Two Carbonyl stretching frequencies were obtained near 1700 cm^{-1} and 1670 cm^{-1} in all the compounds while frequency for thioamide group (>CS) was obtained near 1150 cm^{-1} in DNJ-1701 to DNJ-1705. Frequencies for ether linkage, C-N (3°) and C-N (2°) were obtained near 1050 cm^{-1}, 1350 cm^{-1} and 1320 cm^{-1} respectively in all the compounds. Characteristic frequency for 1, 4-disubstitution (near 820 cm^{-1}) was found in DNJ-1602, DNJ-1604, DNJ-1605, DNJ-1702, DNJ-1704 and DNJ-1705 while for 1, 3-disubstitution (near 770 cm^{-1}) was found in DNJ-1603 and DNJ-1703. Characteristic frequencies for C-Cl (near 870 cm^{-1}), C-F (near 990 cm^{-1}) were found in DNJ-1604, DNJ-1704 and DNJ-1605, DNJ-1705 respectively.

4.11.3 ^{1}H NMR SPECTRAL STUDY

^{1}H & ^{13}C NMR spectra of the synthesized compounds were recorded on **Bruker Avance II 400** spectrometer. Sample solutions were made in $CDCl_3$ solvent using tetramethylsilane (TMS) as the internal standard unless otherwise mentioned. Numbers of protons and numbers of carbons identified from H NMR & C NMR spectrum and their chemical shift (δ ppm) were in the agreement of the structure of the molecule. *J* values were calculated to identify o, m and p coupling. In some cases, aromatic protons were obtained as multiplet. ^{1}H & ^{13}C NMR spectral interpretation can be discussed as under.

^{1}H NMR spectral interpretation of 3-{[3-(4-chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl] methylene}-1-(2, 6-dichlorophenyl)-1, 3-dihydro-2*H*-indol-2-one (DNJ-705)

1. Due to the two chlorine atoms substituted in meta position and nitrogen atom substituted to the para position, one aromatic proton of C_{13} became shielded and gave doublet at 6.45 δ ppm. Due to the same environment of chlorine groups, two protons present at C_{12} and C_{14} became identical and proton of C_{13} gave doublet instead of double doublet.
2. Arylidine proton of C_{17} became deshielded and gave singlet in aromatic region at 7.61 δ ppm.
3. One most deshielded proton of pyrazole ring gave singlet in down field at 9.92 δ ppm.
4. Rests of the peaks are due to the aromatic protons of two phenyl rings substituted in pyrazole ring and two aromatic rings, one substituted at the nitrogen atom of indolinone nucleus and another fused to the nitrogen containing five membered ring.

^{1}H NMR spectral interpretation of 3-{(2*Z*)-3-[3-(4-chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl] prop-2-enoyl}-4-hydroxy-2*H*-chromen-2-one (DNJ-804)

1. Two protons of C_{15} and C_{16} gave double doublet in upfield at 5.70 δ ppm and 5.60 δ ppm respectively. *J* value of both the double doublet was come to 3.6 Hz which proved to be the compound-cis isomer.
2. One proton of hydroxyl group became highest deshielded and did not appear till 10 δ ppm.
3. One proton of pyrazole ring became most deshielded and gave singlet at 7.95 δ ppm.
4. Rests of the peaks are due to the aromatic protons of two phenyl rings substituted in pyrazole ring and one phenyl ring fused to the pyran ring.

^{1}H NMR spectral interpretation of 3-{[3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl] methylene}-2*H*-chromene-2, 4(3*H*)-dione (DNJ-905)

1. Arylidine proton of C_{12} became deshielded and gave single in aromatic region at 7.95 δ ppm.

2. One most deshielded proton of pyrazole ring gave singlet in the down field at 8.24 δ ppm.
3. Rests of the peaks are due to the aromatic protons of two phenyl rings substituted in pyrazole ring and one phenyl ring fused to the pyran ring.

^{1}H NMR spectral interpretation of 4-[3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl]-3, 4-dihydro-2*H*-chromeno [4, 3-d] pyrimidine-2, 5(1*H*)-dione (DNJ-1603)

1. Two most deshielded protons of secondary amine groups of pyrimidine ring gave singlet at 9.71 δ ppm and 9.97 δ ppm for C_7 and C_5 respectively.
2. One deshielded proton of pyrazole ring gave singlet in the down field at 8.24 δ ppm.
3. One shielded proton of pyrimidine ring (C_8) gave singlet at 6.53 δ ppm.
4. Rests of the peaks are due to the aromatic protons of two phenyl rings substituted in pyrazole ring and one phenyl ring fused to the pyran ring.

^{1}H NMR spectral interpretation of 4-[3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl]-2-thioxo-1,2,3,4-tetrahydro-5*H*-chromeno [4, 3-d] pyrimidin-5-one (DNJ-1703)

1. Two most deshielded protons of secondary amine groups of pyrimidine ring gave singlet at 9.79 δ ppm and 10.00 δ ppm for C_7 and C_5 respectively.
2. One deshielded proton of pyrazole ring gave singlet in the down field at 8.24 δ ppm.
3. One shielded proton of pyrimidine ring (C_8) gave singlet at 6.53 δ ppm.
4. Rests of the peaks are due to the aromatic protons of two phenyl rings substituted in pyrazole ring and one phenyl ring fused to the pyran ring.

4.11.4 ELEMENTAL ANALYSIS

Elemental analysis of the synthesized compounds was carried out on **Vario EL Carlo Erba 1108** which showed calculated and found percentage values of Carbon, Hydrogen and Nitrogen in support of the structure of synthesized compounds. The spectral and elemental analysis data are given for individual compounds.

4.12 ANALYTICAL DATA

1-(2, 6-DICHLORO PHENYL)-3-{[3-(4-NITRO PHENYL)-1-PHENYL-1*H*-PYRAZOL-4-YL] METHYLENE}-1, 3-DIHYDRO-2*H*-INDOL-2-ONE (DNJ-701): IR (KBr, cm^{-1}): 3063 (Ar-H, str), 1702 (>CO), 1602, 1564, 1523, 1493 (Ar-H, ben), 1334 (C-N, Ar, 3°), 885 (C-Cl), 830 (1,4-di sub), 748 (1,2-di sub); MS m/z = 553 (M^+); Anal. Calcd. for $C_{30}H_{18}Cl_2N_4O_3$: C, 65.11; H, 3.28; N, 10.12. Found: C, 65.15; H, 3.26; N, 10.15.

1-(2, 6-DICHLORO PHENYL)-3-{[3-(3-NITRO PHENYL)-1-PHENYL-1*H*-PYRAZOL-4-YL] METHYLENE}-1, 3-DIHYDRO-2*H*-INDOL-2-ONE (DNJ-702): IR (KBr, cm^{-1}): 3060 (Ar-H, str), 2962 ($-CH_2$), 2856 ($-CH_3$), 1703 (>CO), 1605, 1560, 1521, 1493 (Ar-H, ben), 1333 (C-N, Ar, 3°), 890 (C-Cl), 775 (1,3-di sub), 750 (1,2-di sub); MS m/z = 553 (M^+); Anal. Calcd. for $C_{30}H_{18}Cl_2N_4O_3$: C, 65.11; H, 3.28; N, 10.12. Found: C, 65.08; H, 3.24; N, 10.10.

1-(2, 6-DICHLORO PHENYL)-3-[(1, 3-DIPHENYL-1*H*-PYRAZOL-4-YL) METHYLENE]-1, 3-DIHYDRO-2*H*-INDOL-2-ONE (DNJ-703): IR (KBr, cm^{-1}): 3066 (Ar-H, str), 2962 ($-CH_2$), 2856 ($-CH_3$), 1705 (>CO), 1605, 1564, 1523, 1493 (Ar-H, ben), 1335 (C-N, Ar, 3°), 892 (C-Cl), 748 (1,2-di sub); MS m/z = 508 (M^+); Anal. Calcd. for $C_{30}H_{19}Cl_2N_3O$: C, 70.87; H, 3.77; N, 8.27. Found: C, 70.90; H, 3.80; N, 8.30.

1-(2, 6-DICHLORO PHENYL)-3-{[3-(4-FLUORO PHENYL)-1-PHENYL-1*H*-PYRAZOL-4-YL] METHYLENE}-1, 3-DIHYDRO-2*H*-INDOL-2-ONE (DNJ-704): IR (KBr, cm^{-1}): 3067 (Ar-H, str), 2966 (-CH_2), 2860 (-CH_3), 1700 (>CO), 1600, 1565, 1525, 1491 (Ar-H, ben), 1335 (C-N, Ar, 3°), 990 (C-F), 890 (C-Cl), 831 (1,4-di sub), 752 (1,2-di sub); MS m/z = 526 (M^+); Anal. Calcd. for $C_{30}H_{18}Cl_2FN_3O$: C, 68.45; H, 3.45; N, 7.98. Found: C, 68.50; H, 3.50; N, 8.01.

3-{[3-(4-CHLOROPHENYL)-1-PHENYL-1*H*-PYRAZOL-4-YL] METHYLENE}-1-(2, 6-DICHLORO PHENYL)-1, 3-DIHYDRO-2*H*-INDOL-2-ONE (DNJ-705): IR (KBr, cm^{-1}): 3057 (Ar-H, str), 1700 (>CO), 1602, 1564, 1523, 1493 (Ar-H, ben), 1330 (C-N, Ar, 3°), 888 (C-Cl), 834 (1,4-di sub), 746 (1,2-di sub); ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) 6.45 (1H, d, *J* = 7.72 Hz), 7.12 (1H, m, *J* = 0.84 Hz, *J* = 0.64 Hz, *J* = 0.80 Hz), 7.22 (1H, m, *J* = 0.88 Hz, *J* = 0.88 Hz, *J* = 0.92 Hz), 7.33 (1H, t), 7.46 (3H, m, *J* = 1.0 Hz, *J* = 1.88 Hz), 7.55 (5H, m, *J* = 3.16 Hz, *J* = 1.92 Hz), 7.61 (1H, s), 7.69 (1H, m, *J* = 1.84 Hz), 7.87 (2H, t, *J* = 1.16 Hz), 9.92 (1H, s); MS m/z = 542 (M^+); Anal. Calcd. for $C_{30}H_{18}Cl_3N_3O$: C, 66.38; H, 3.34; N, 7.74. Found: C, 66.33; H, 3.30; N, 7.70.

3-[(2*Z*)-3-(1, 3-DIPHENYL-1*H*-PYRAZOL-4-YL) PROP-2-ENOYL]-4-HYDROXY-2*H*-CHROMEN-2-ONE (DNJ-801): IR (KBr, cm^{-1}): 3551 (-OH), 3077 (Ar-H, str), 2971 (-CH_3), 2858 (-CH_2), 1727 (>CO), 1700 (>CO), 1621, 1575, 1500, 1435 (Ar-H, ben), 1317 (C-N, Ar, 3°), 1088 (C-O-C); MS m/z = 434 (M^+); Anal. Calcd. for $C_{27}H_{18}N_2O_4$: C, 74.64; H, 4.18; N, 6.45. Found: C, 74.69; H, 4.22; N, 6.50.

4-HYDROXY-3-{(2*Z*)-3-[3-(4-NITRO PHENYL)-1-PHENYL-1*H*-PYRAZOL-4-YL] PROP-2-ENOYL}-2*H*-CHROMEN-2-ONE (DNJ-802): 3567 (-OH), 3070 (Ar-H, str), 2969 (-CH_3), 2863 (-CH_2), 1720 (>CO), 1703 (>CO), 1617, 1571, 1495, 1420 (Ar-H, ben), 1320 (C-N, Ar, 3°), 1081 (C-O-C), 831 (1,4-di sub); MS m/z = 479 (M^+); Anal. Calcd. for $C_{27}H_{17}N_3O_6$: C, 67.64; H, 3.57; N, 8.76. Found: C, 67.67; H, 3.60; N, 8.72.

4-HYDROXY-3-{(2*Z*)-3-[3-(3-NITRO PHENYL)-1-PHENYL-1*H*-PYRAZOL-4-YL] PROP-2-ENOYL}-2*H*-CHROMEN-2-ONE (DNJ-803): 3577 (-OH), 3066

(Ar-H, str), 2971 (-CH_3), 2853 (-CH_2), 1724 (>CO), 1708 (>CO), 1625, 1580, 1535, 1440 (Ar-H, ben), 1327 (C-N, Ar, 3°), 1092 (C-O-C), 787 (1,3-di sub); MS m/z = 479 (M^+); Anal. Calcd. for $C_{27}H_{17}N_3O_6$: C, 67.64; H, 3.57; N, 8.76. Found: C, 67.69; H, 3.53; N, 8.80.

3-{(2*Z*)-3-[3-(4-CHLORO PHENYL)-1-PHENYL-1*H*-PYRAZOL-4-YL] PROP-2-ENOYL}-4-HYDROXY-2*H*-CHROMEN-2-ONE (DNJ-804): IR (KBr, cm^{-1}): 3539 (-OH), 1722 (>CO), 1708 (>CO), 1610, 1505, 1429 (Ar-H, ben), 1305 (C-N, Ar, 3°), 1095 (C-O-C), 876 (C-Cl), 842 (1,4-di sub); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 5.60 (1H, d, *J* = 3.6 Hz), 5.70 (1H, d, *J* = 3.6 Hz), 7.28 (4H, q), 7.45 (2H, t), 7.53 (2H, q, *J* = 0.88 Hz, *J* = 0.92 Hz), 7.68 (2H, d, *J* = 9.52 Hz), 7.86 (3H, qt, *J* = 1.92 Hz), 7.95 (1H, s); MS m/z = 468 (M^+); Anal. Calcd. for $C_{27}H_{17}ClN_2O_4$: C, 69.16; H, 3.65; N, 5.97. Found: C, 69.20; H, 3.60; N, 6.00.

3-{(2*Z*)-3-[3-(4-FLUORO PHENYL)-1-PHENYL-1*H*-PYRAZOL-4-YL] PROP-2-ENOYL}-4-HYDROXY-2*H*-CHROMEN-2-ONE (DNJ-805): IR (KBr, cm^{-1}): 3547 (-OH), 3060 (Ar-H, str), 2958 (-CH_3), 2870 (-CH_2), 1725 (>CO), 1712 (>CO), 1605, 1500, 1470, 1429 (Ar-H, ben), 1310 (C-N, Ar, 3°), 1092 (C-O-C), 995 (C-F), 837 (1,4-di sub); MS m/z = 452 (M^+); Anal. Calcd. for $C_{27}H_{17}FN_2O_4$: C, 71.68; H, 3.79; N, 6.19. Found: C, 71.70; H, 3.80; N, 6.15.

3-[(1, 3-DIPHENYL-1*H*-PYRAZOL-4-YL) METHYLENE]-2*H*-CHROMENE-2, 4(3*H*)-DIONE (DNJ-901): IR (KBr, cm^{-1}): 2966 (-CH_3), 2866 (-CH_2), 1732 (>CO, lactam ring), 1666 (>CO), 1608, 1560, 1508, 1470 (Ar-H, ben), 1380 (C-N, Ar, 3°), 1115 (C-O-C); MS m/z = 392 (M^+); Anal. Calcd. for $C_{25}H_{16}N_2O_3$: C, 76.52; H, 4.11; N, 7.14. Found: C, 76.57; H, 4.15; N, 7.18.

3-{[3-(4-NITROPHENYL)-1-PHENYL-1*H*-PYRAZOL-4-YL] METHYLENE}-2*H*-CHROMENE-2, 4(3*H*)-DIONE (DNJ-902): IR (KBr, cm^{-1}): 2959 (-CH_3), 2866 (-CH_2), 1730 (>CO, lactam ring), 1670 (>CO), 1610, 1560, 1503, 1455 (Ar-H, ben), 1385 (C-N, Ar, 3°), 1105 (C-O-C), 840 (1,4-di sub); MS m/z = 437 (M^+); Anal. Calcd. for $C_{25}H_{15}N_3O_5$: C, 68.65; H, 3.46; N, 9.61. Found: C, 68.60; H, 3.50; N, 9.65.

3-{[3-(3-NITROPHENYL)-1-PHENYL-1*H*-PYRAZOL-4-YL] METHYLENE}-2*H*-CHROMENE-2,4(3*H*)-DIONE (DNJ-903): IR (KBr, cm^{-1}): 2967 ($-CH_3$), 2855 ($-CH_2$), 1725 (>CO, lactam ring), 1665 (>CO), 1603, 1568, 1515, 1460 (Ar-H, ben), 1381 (C-N, Ar, 3°), 1107 (C-O-C), 781 (1,3-di sub); MS m/z = 437 (M^+); Anal. Calcd. for $C_{25}H_{15}N_3O_5$: C, 68.65; H, 3.46; N, 9.61. Found: C, 68.66; H, 3.42; N, 9.57.

3-{[3-(4-CHLORO PHENYL)-1-PHENYL-1*H*-PYRAZOL-4-YL] METHYLENE}-2*H*-CHROMENE-2, 4(3*H*)-DIONE (DNJ-904): IR (KBr, cm^{-1}): 2960 ($-CH_3$), 2866 ($-CH_2$), 1721 (>CO, lactam ring), 1667 (>CO), 1606, 1563, 1500, 1460 (Ar-H, ben), 1385 (C-N, Ar, 3°), 1110 (C-O-C), 877 (C-Cl), 836 (1,4-di sub); MS m/z = 426 (M^+); Anal. Calcd. for $C_{25}H_{15}ClN_2O_3$: C, 70.34; H, 3.54; N, 6.56. Found: C, 70.30; H, 3.58; N, 6.60.

3-{[3-(4-FLUORO PHENYL)-1-PHENYL-1*H*-PYRAZOL-4-YL] METHYLENE}-2*H*-CHROMENE-2, 4(3*H*)-DIONE (DNJ-905): IR (KBr, cm^{-1}): 1727 (>CO, lactam ring), 1660 (>CO), 1612, 1560, 1505, 1464 (Ar-H, ben), 1382 (C-N, Ar, 3°), 1112 (C-O-C), 998 (C-F), 839 (1,4-di sub); ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.28 (4H, q), 7.45 (3H, t), 7.53 (3H, q, *J* = 1.28 Hz, *J* = 1.24 Hz), 7.86 (3H, qt, *J* = 1.92 Hz), 7.95 (1H, s), 8.24 (1H, s); MS m/z = 410 (M^+); Anal. Calcd. for $C_{25}H_{15}FN_2O_3$: C, 73.17; H, 3.68; N, 6.83. Found: C, 73.20; H, 3.72; N, 6.87.

4-(1, 3-DIPHENYL-1*H*-PYRAZOL-4-YL)-3, 4-DIHYDRO-2*H*-CHROMENO [4, 3-*D*] PYRIMIDINE-2, 5(1*H*)-DIONE (DNJ-1601): IR (KBr, cm^{-1}): 3185 (-NH), 3095 (Ar-H, str), 2967 ($-CH_3$), 2847 ($-CH_2$), 1727 (>CO, lactam ring), 1655 (>CO, amide), 1610, 1562, 1544, 1493 (Ar-H, ben), 1347 (C-N, Ar, 3°), 1315 (C-N, Ar, 2°), 1057 (C-O-C); MS m/z = 434 (M^+); Anal. Calcd. for $C_{26}H_{18}N_4O_3$: C, 71.88; H, 4.18; N, 12.90. Found: C, 71.83; H, 4.22; N, 12.94.

4-[3-(4-NITRO PHENYL)-1-PHENYL-1*H*-PYRAZOL-4-YL]-3, 4-DIHYDRO-2*H*-CHROMENO [4, 3-*D*] PYRIMIDINE-2, 5(1*H*)-DIONE (DNJ-1602): IR (KBr, cm^{-1}): 3182 (-NH), 3090 (Ar-H, str), 2962 ($-CH_3$), 2850 ($-CH_2$), 1730 (>CO, lactam ring), 1660 (>CO, amide), 1604, 1560, 1550, 1500 (Ar-H, ben), 1350

(C-N, Ar, 3°), 1320 (C-N, Ar, 2°), 1055 (C-O-C), 820 (1,4-di sub); MS m/z = 479 (M^+); Anal. Calcd. for $C_{26}H_{17}N_5O_5$: C, 65.13; H, 3.57; N, 14.61. Found: C, 65.17; H, 3.60; N, 14.65.

4-[3-(3-NITROPHENYL)-1-PHENYL-1*H*-PYRAZOL-4-YL]-3, 4-DIHYDRO-2*H*-CHROMENO [4, 3-*D*] PYRIMIDINE-2, 5(1*H*)-DIONE (DNJ-1603): IR (KBr, cm^{-1}): 3180 (-NH), 3092 (Ar-H, str), 1729 (>CO, lactam ring), 1657 (>CO, amide), 1611, 1565, 1548, 1498 (Ar-H, ben), 1351 (C-N, Ar, 3°), 1318 (C-N, Ar, 2°), 1050 (C-O-C), 780 (1,3-di sub); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 6.53 (1H, s), 7.28 (3H, q), 7.45 (2H, t), 7.53 (2H, q, *J* = 0.88 Hz, *J* = 0.68 Hz), 7.68 (2H, d, *J* = 8.88 Hz), 7.86 (3H, qt, *J* = 0.92 Hz), 7.95 (1H, s), 8.24 (1H, s), 9.71 (1H, s), 9.97 (1H, s); MS m/z = 479 (M^+); Anal. Calcd. for $C_{26}H_{17}N_5O_5$: C, 65.13; H, 3.57; N, 14.61. Found: C, 65.10; H, 3.53; N, 14.57.

4-[3-(4-CHLORO PHENYL)-1-PHENYL-1*H*-PYRAZOL-4-YL]-3, 4-DIHYDRO-2*H*-CHROMENO [4, 3-*D*] PYRIMIDINE-2, 5(1*H*)-DIONE (DNJ-1604): IR (KBr, cm^{-1}): 3189 (-NH), 3087 (Ar-H, str), 2958 ($-CH_3$), 2867 ($-CH_2$), 1725 (>CO, lactam ring), 1655 (>CO, amide), 1605, 1560, 1542, 1488 (Ar-H, ben), 1355 (C-N, Ar, 3°), 1321 (C-N, Ar, 2°), 1047 (C-O-C), 875 (C-Cl), 825 (1,4-di sub); MS m/z = 468 (M^+); Anal. Calcd. for $C_{26}H_{17}ClN_4O_3$: C, 66.60; H, 3.65; N, 11.95. Found: C, 66.65; H, 3.70; N, 12.00.

4-[3-(4-FLUORO PHENYL)-1-PHENYL-1*H*-PYRAZOL-4-YL]-3, 4-DIHYDRO-2*H*-CHROMENO [4, 3-*D*] PYRIMIDINE-2, 5(1*H*)-DIONE (DNJ-1605): IR (KBr, cm^{-1}): 3182 (-NH), 3080 (Ar-H, str), 2952 ($-CH_3$), 2861 ($-CH_2$), 1727 (>CO, lactam ring), 1659 (>CO, amide), 1601, 1555, 1530, 1480 (Ar-H, ben), 1350 (C-N, Ar, 3°), 1320 (C-N, Ar, 2°), 1052 (C-O-C), 995 (C-F), 830 (1,4-di sub); MS m/z = 452 (M^+); Anal. Calcd. for $C_{26}H_{17}FN_4O_3$: C, 69.02; H, 3.79; N, 12.38. Found: C, 68.98; H, 3.82; N, 12.34.

4-(1, 3-DIPHENYL-1*H*-PYRAZOL-4-YL)-5-THIOXO-1, 3, 4, 5-TETRAHYDRO-2*H*-CHROMENO [4, 3-*D*] PYRIMIDIN-2-ONE (DNJ-1701): IR (KBr, cm^{-1}): 3187 (-NH), 3092 (Ar-H, str), 2965 ($-CH_3$), 2841 ($-CH_2$), 1725 (>CO, lactam ring), 1608, 1565, 1541, 1490 (Ar-H, ben), 1355 (C-N, Ar, 3°),

1315 (C-N, Ar, 2°), 1167 (>CS, thioamide), 1085 (C-O-C); MS m/z = 450 (M^+); Anal. Calcd. for $C_{26}H_{18}N_4O_2S$: C, 69.32; H, 4.03; N, 12.44. Found: C, 69.36; H, 4.08; N, 12.48.

4-[3-(4-NITRO PHENYL)-1-PHENYL-1*H*-PYRAZOL-4-YL]-5-THIOXO-1, 3, 4, 5-TETRAHYDRO-2*H*-CHROMENO [4, 3-*D*] PYRIMIDIN-2-ONE (DNJ-1702): IR (KBr, cm^{-1}): 3187 (-NH), 3085 (Ar-H, str), 2964 ($-CH_3$), 2855 ($-CH_2$), 1727 (>CO, lactam ring), 1615, 1568, 1545, 1494 (Ar-H, ben), 1355 (C-N, Ar, 3°), 1326 (C-N, Ar, 2°), 1165 (>CS, thioamide), 1085 (C-O-C), 827 (1,4-di sub); MS m/z = 495 (M^+); Anal. Calcd. for $C_{26}H_{17}N_5O_4S$: C, 63.02; H, 3.46; N, 14.13. Found: C, 63.06; H, 3.41; N, 14.17.

4-[3-(3-NITRO PHENYL)-1-PHENYL-1*H*-PYRAZOL-4-YL]-5-THIOXO-1, 3, 4, 5-TETRAHYDRO-2*H*-CHROMENO [4, 3-*D*] PYRIMIDIN-2-ONE (DNJ-1703): IR (KBr, cm^{-1}): 3178 (-NH), 3091 (Ar-H, str), 1729 (>CO, lactam ring), 1609, 1561, 1539, 1501 (Ar-H, ben), 1353 (C-N, Ar, 3°), 1316 (C-N, Ar, 2°), 1162 (>CS, thioamide), 1088 (C-O-C), 750 (1,3-di sub); 6.53 (1H, s), 7.28 (3H, q), 7.45 (2H, t), 7.53 (2H, q, *J* = 0.88 Hz, *J* = 1.24 Hz), 7.68 (2H, d, *J* = 9.12 Hz), 7.86 (3H, qt, *J* = 1.96 Hz), 7.95 (1H, s), 8.24 (1H, s), 9.79 (1H, s), 10.00 (1H, s); MS m/z = 495 (M^+); Anal. Calcd. for $C_{26}H_{17}N_5O_4S$: C, 63.02; H, 3.46; N, 14.13. Found: C, 63.04; H, 3.42; N, 14.17.

4-[3-(4-CHLORO PHENYL)-1-PHENYL-1*H*-PYRAZOL-4-YL]-5-THIOXO-1, 3, 4, 5-TETRAHYDRO-2*H*-CHROMENO [4, 3-*D*] PYRIMIDIN-2-ONE (DNJ-1704): IR (KBr, cm^{-1}): 3182 (-NH), 3085 (Ar-H, str), 2961 ($-CH_3$), 2865 ($-CH_2$), 1729 (>CO, lactam ring), 1607, 1565, 1545, 1492 (Ar-H, ben), 1353 (C-N, Ar, 3°), 1325 (C-N, Ar, 2°), 1160 (>CS, thioamide), 1047 (C-O-C), 877 (C-Cl), 821 (1,4-di sub); MS m/z = 484 (M^+); Anal. Calcd. for $C_{26}H_{17}ClN_4O_2S$: C, 64.39; H, 3.53; N, 11.55. Found: C, 64.35; H, 3.57; N, 11.50.

4-[3-(4-FLUORO PHENYL)-1-PHENYL-1*H*-PYRAZOL-4-YL]-5-THIOXO-1, 3, 4, 5-TETRAHYDRO-2*H*-CHROMENO [4, 3-*D*] PYRIMIDIN-2-ONE (DNJ-1705): IR (KBr, cm^{-1}): 3188 (-NH), 3085 (Ar-H, str), 2951 ($-CH_3$), 2863 ($-CH_2$), 1730 (>CO, lactam ring), 1613, 1559, 1535, 1487 (Ar-H, ben), 1357 (C-N, Ar,

3°), 1328 (C-N, Ar, 2°), 1164 (>CS, thioamide), 1052 (C-O-C), 990 (C-F), 832 (1,4-di sub); MS m/z = 468 (M^+); Anal. Calcd. for $C_{26}H_{17}FN_4O_2S$: C, 66.65; H, 3.66; N, 11.96. Found: C, 66.70; H, 3.70; N, 12.00.

4.13 RESULTS AND DISCUSSION

Four different schemes had been adopted to build up this chapter. This chapter is mostly related to modification in the previous work done by our group as well as others but the scaffolds reported here are new. Earlier indolinone derivatives were known to be prepared by conventional method using piperidine (base) in refluxing methanol or ethanol. Different 2-indolinone derivatives have been prepared by adopting the conventional method. Moreover, microwave assisted method was also employed to compare both the methods in order to acquire best results. Microwave assisted method found much faster than the conventional one and % yield found to be higher than conventional but the purity was similar in both the methods.

It is very well known that C_3 position of 4-hydroxycoumarin is highly reactive. 3-acetyl-4-hydroxycoumarin was synthesized by acetylation on 4-hydroxycoumarin adopting cited literature method. Chalcones of 3-acetyl-4-hydroxycoumarin were prepared using substituted benzaldehydes using piperidine (base) and chloroform which showed good anti viral activity [j]. In this chapter, continuing previous work chalcones of 3-acetyl-4-hydroxycoumarin using differently substituted pyrazole aldehydes were synthesized using the same experimental protocol.

4-hydroxycoumarin and different pyrazole aldehydes were refluxed in methanol under basic condition in order to prepare coumarin dimers but the 4-hydroxycoumarin tautomarize into 2*H*-chromene-2, 4(3*H*)-dione and due to the in situ generation of active methylene group it gave arylidine at C_3-position.

Three component Biginelli reaction was used to prepare the coumarin fused pyrimidine derivatives. Mixture of urea / thio urea, pyrazole aldehyde

[j] J. C. Trivedi, J. B. Bariwal, K. D. Upadhyay, Y. T. Naliapara, S. K. Joshi, C. C. Pannecouque, E. De Clercq and A. K. Shah; *Tet. Lett.*, **2007**, *48(48)*, 8472.

and 4-hydroxycoumarin was refluxed into methanol under acidic condition to give rise to the expected product.

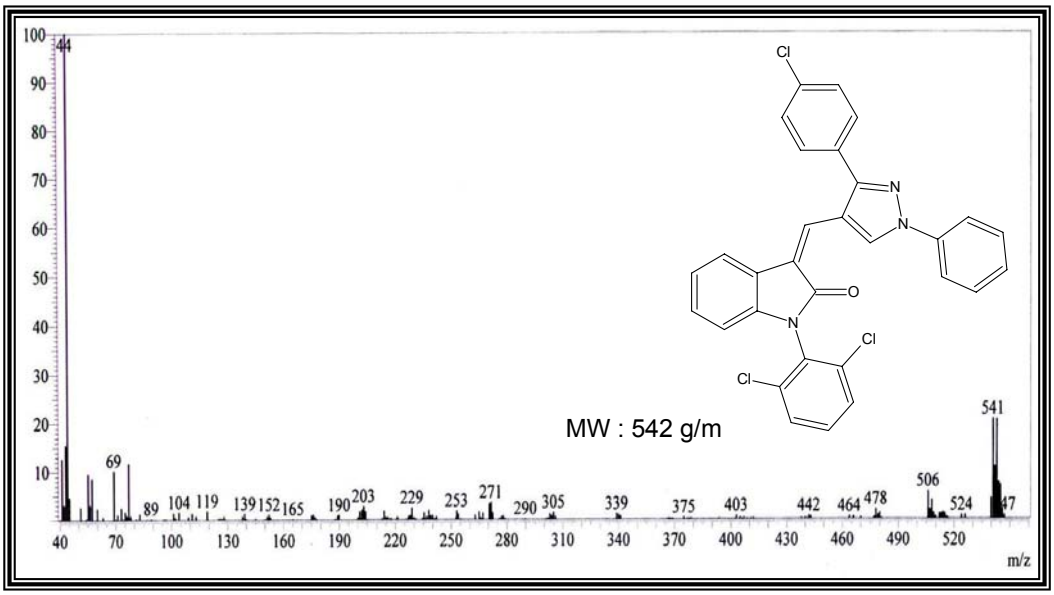
4.14 CONCLUSION

2-Indolinone derivatives have recently been established as an anti cancer compounds and more specifically as tyrosine kinase inhibitors (*SU5416*, *SU5614*, *SU6668*, *SU6597*, *SU6663* and *SU6561*) that block kit activation and growth of small cell in lung cancer. Moreover Virsodia *et. al.* [a] reported the anticancer evaluation results of the arylidine products of 1-(2, 6-dichlorophenyl)-2-indolinone. Biological importance of such scaffolds inspired us to prepare new indolinone derivatives and to carry out their anticancer evaluation.

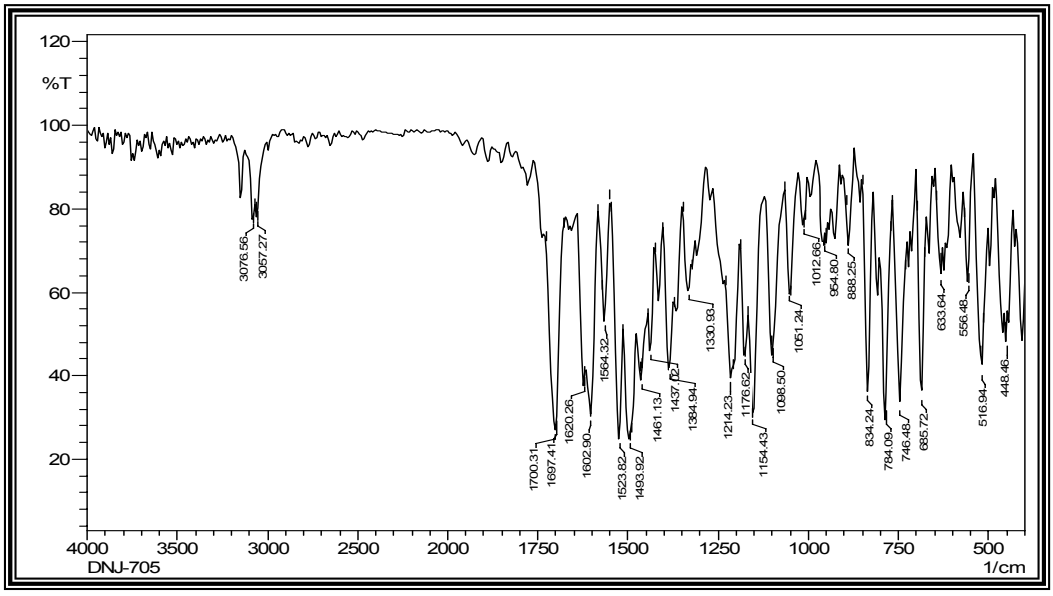
4-hydroxycoumarin is a versatile scaffold and is being consistently used as a building block in organic chemistry as well as in heterocyclic chemistry for the synthesis of different heterocycles. The synthetic versatility of 4-hydroxycoumarin has led to the extensive use of this compound in organic synthesis. 4-hydroxy coumarin shows diversified chemical reactivity and biological profile. Thus it was of interest to study the biological activities of newly synthesized coumarin derivatives.

4.15 SPECTRAL REPRESENTATION OF SYNTHESIZED COMPOUNDS

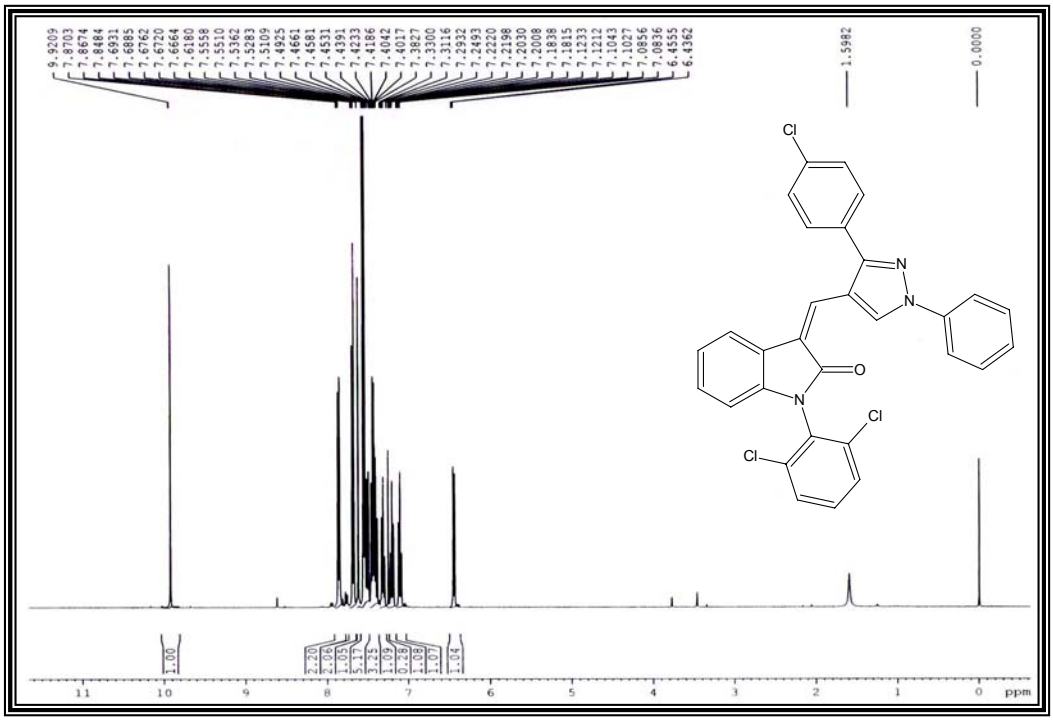
4.15.1 Mass Spectrum of DNJ-705

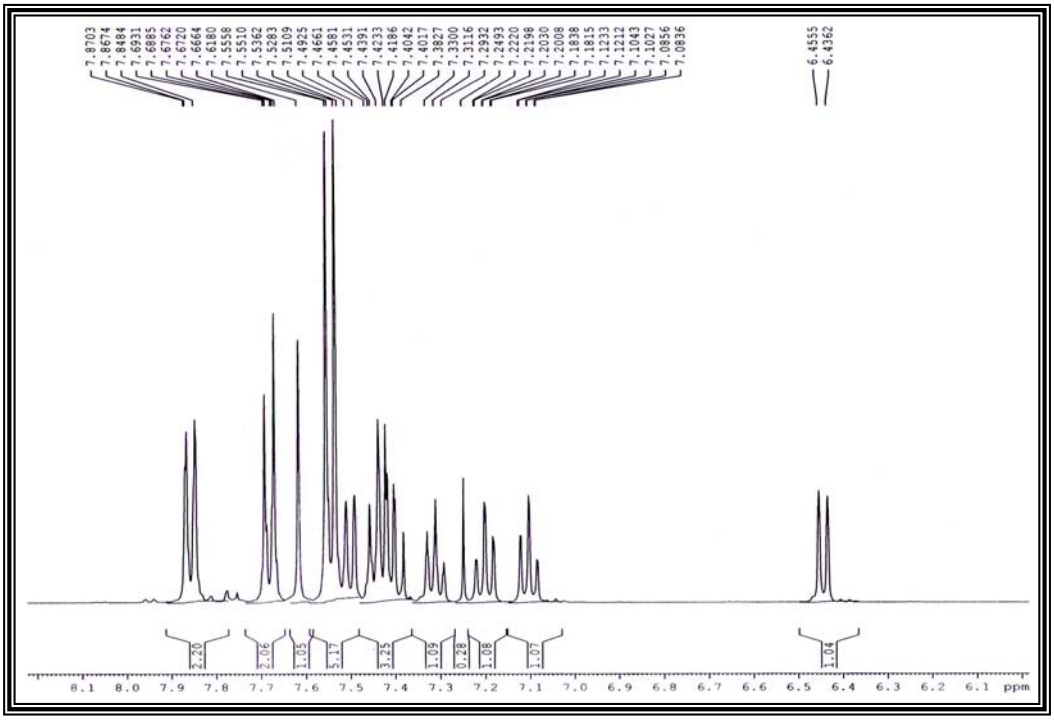


4.15.2 IR Spectrum of DNJ-705

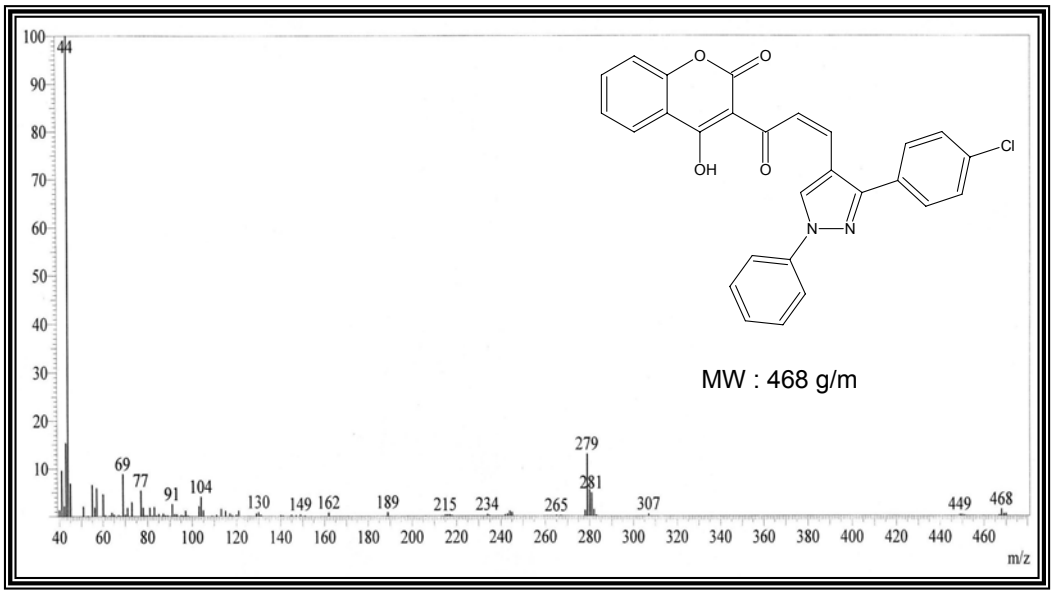


4.15.3 ^{1}H NMR Spectrums of DNJ-705

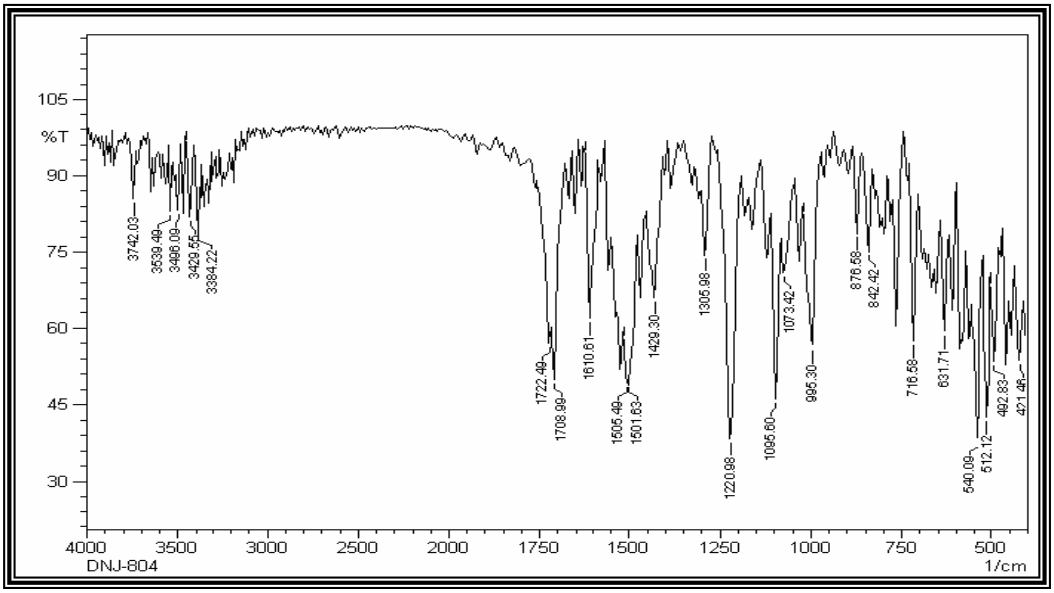




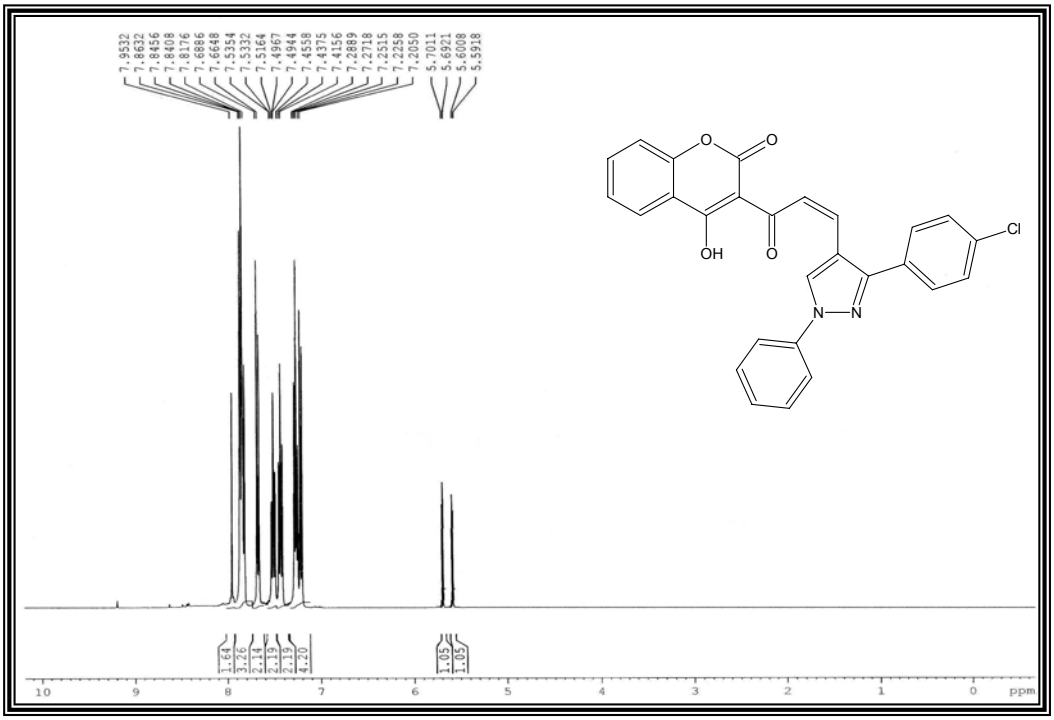
4.15.4 Mass Spectrum of DNJ-804

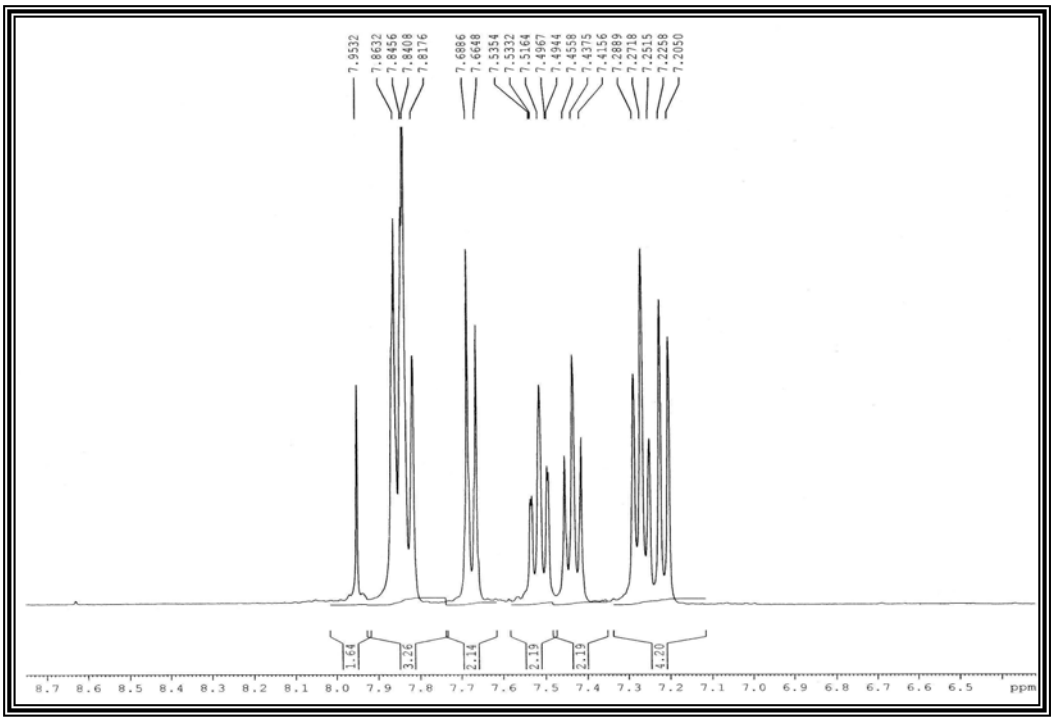


4.15.5 IR Spectrum of DNJ-804

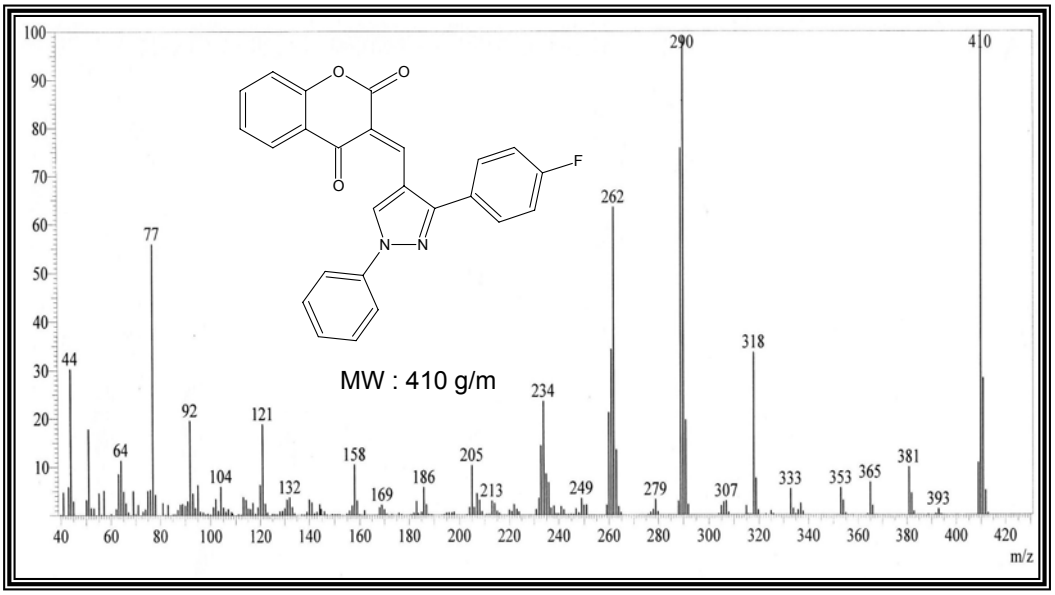


4.15.6 ^{1}H NMR Spectrums of DNJ-804

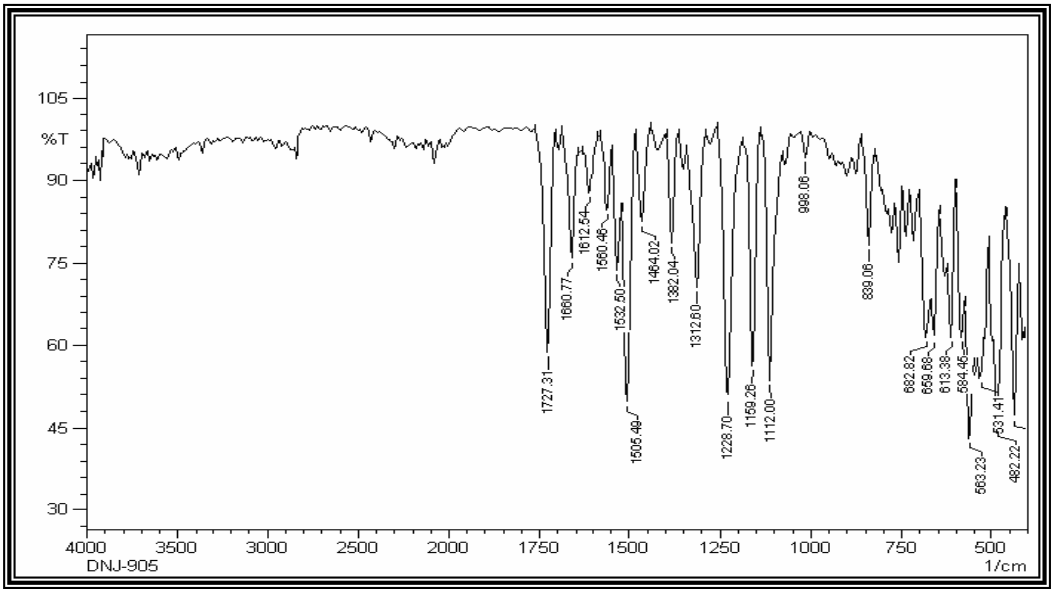




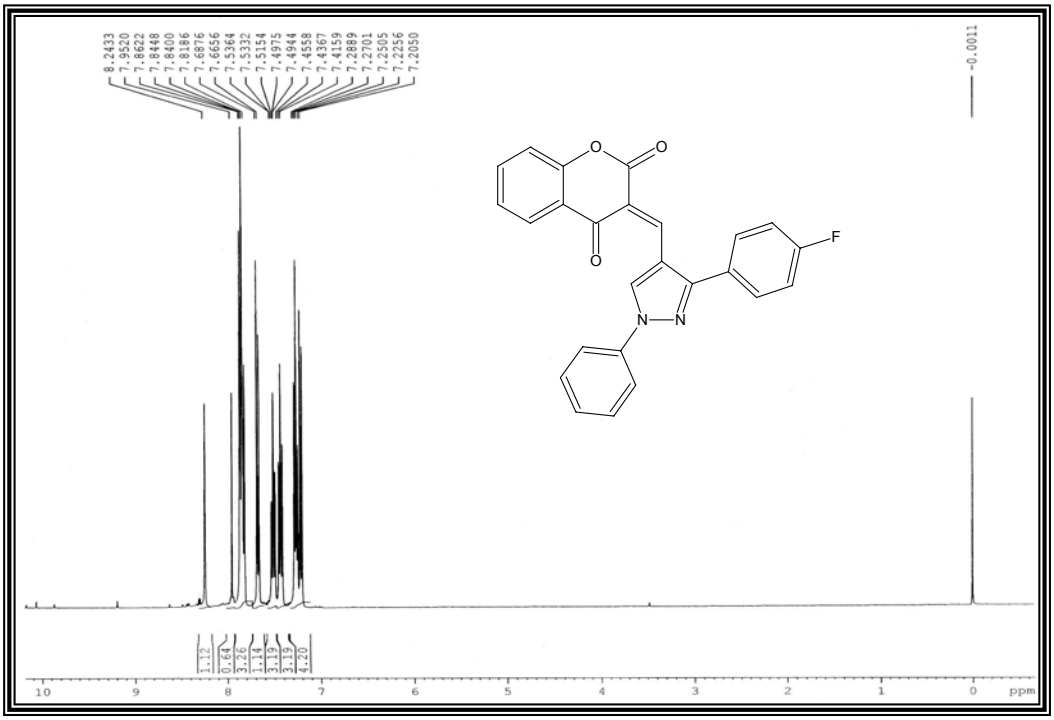
4.15.7 Mass Spectrum of DNJ-905

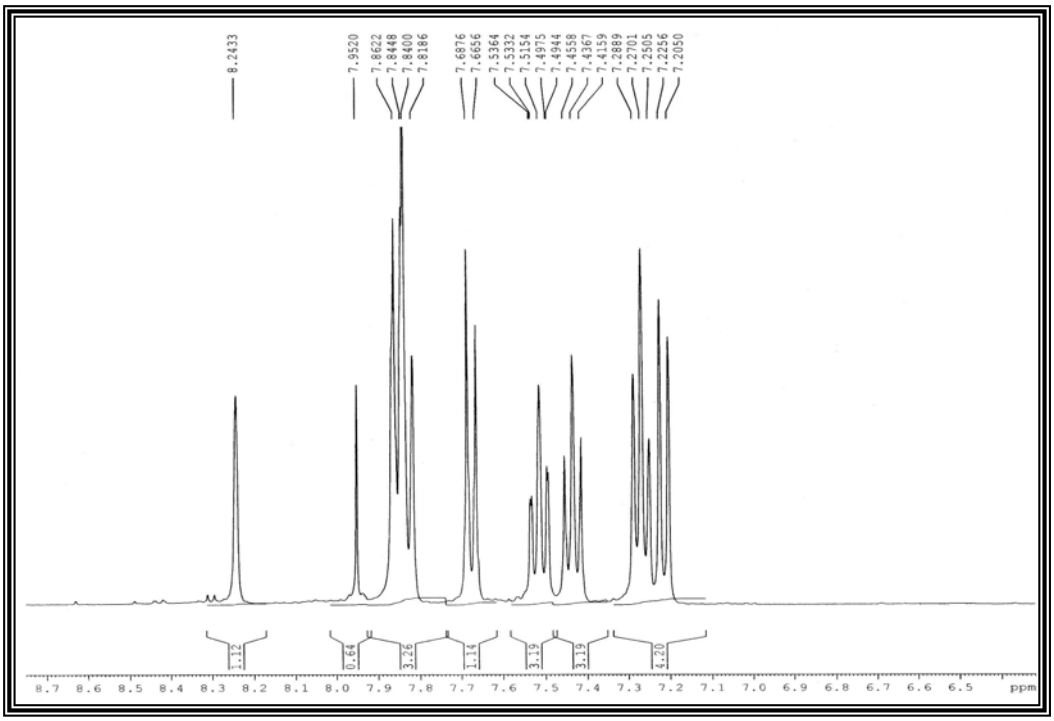


4.15.8 IR Spectrum of DNJ-905

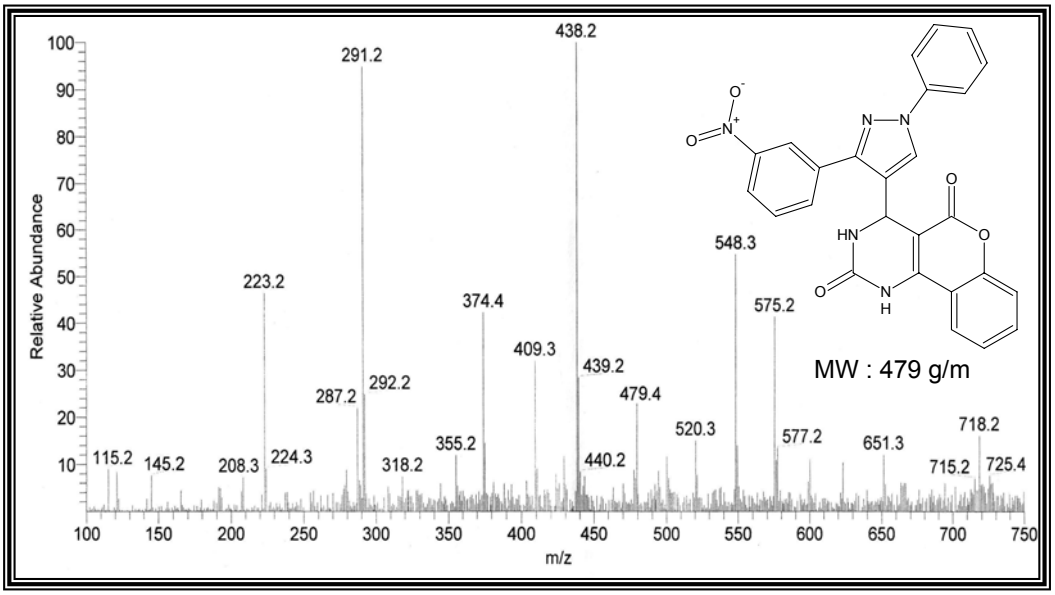


4.15.9 ^{1}H NMR Spectrums of DNJ-905

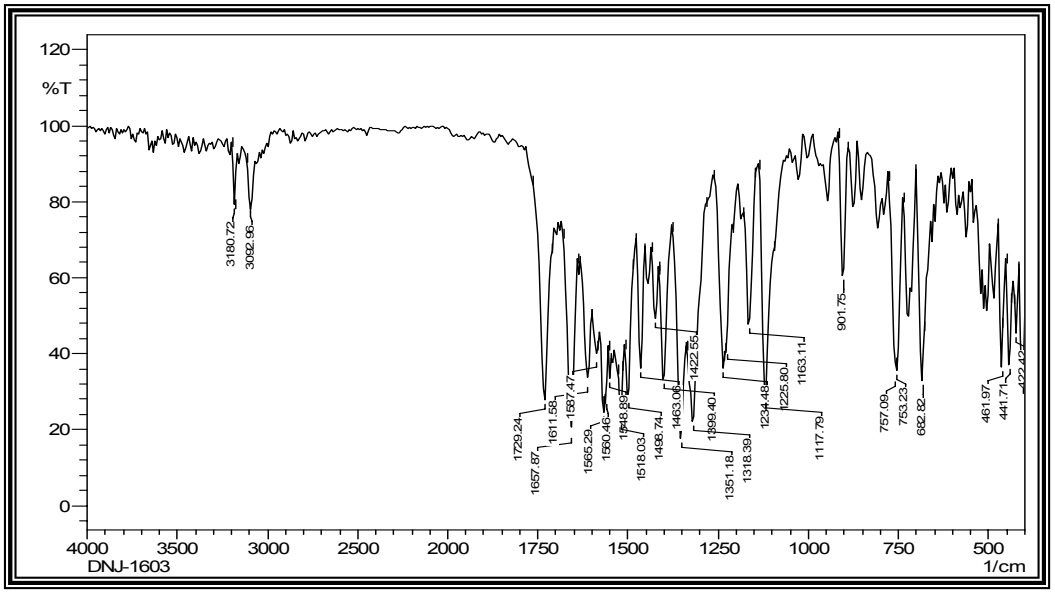




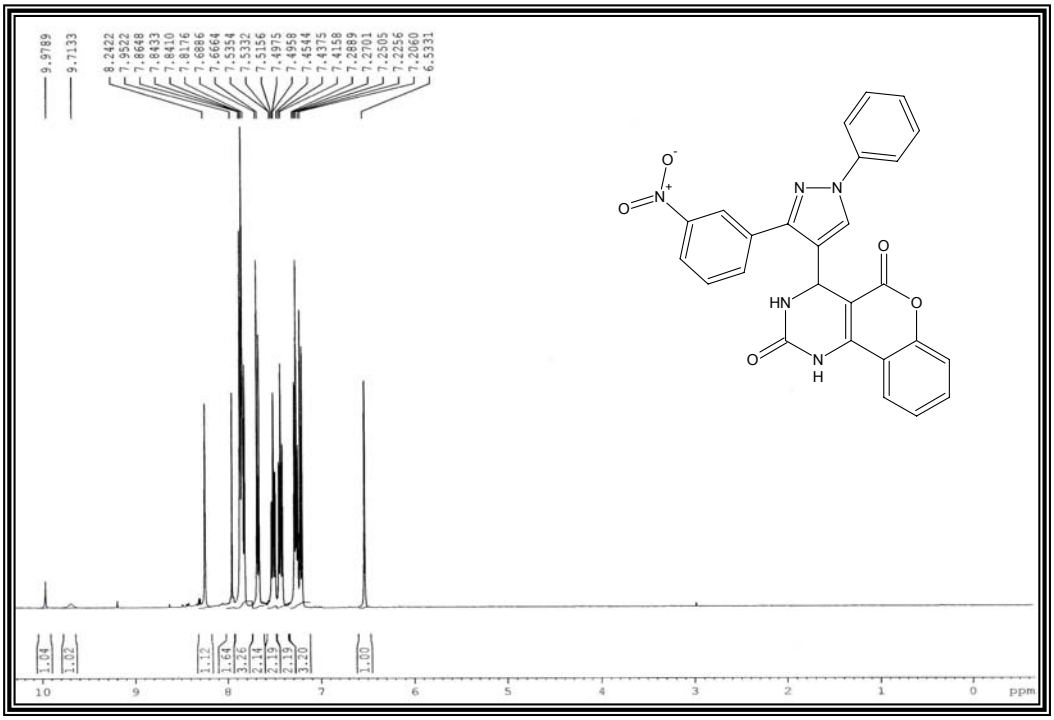
4.15.10 Mass Spectrum of DNJ-1603

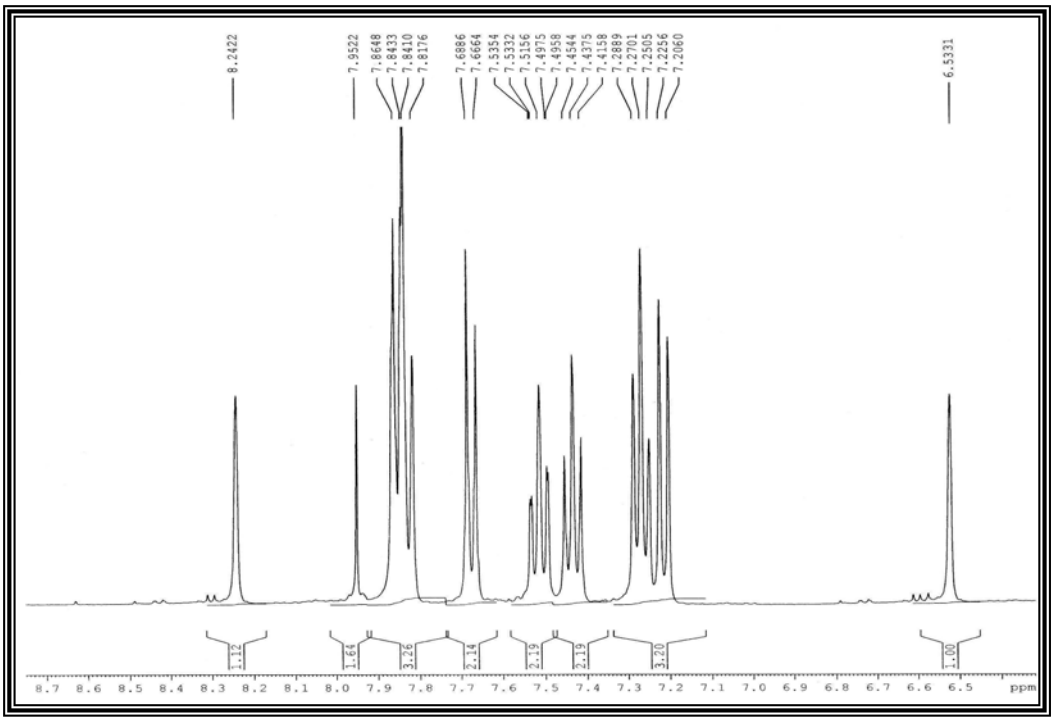


4.15.11 IR Spectrum of DNJ-1603

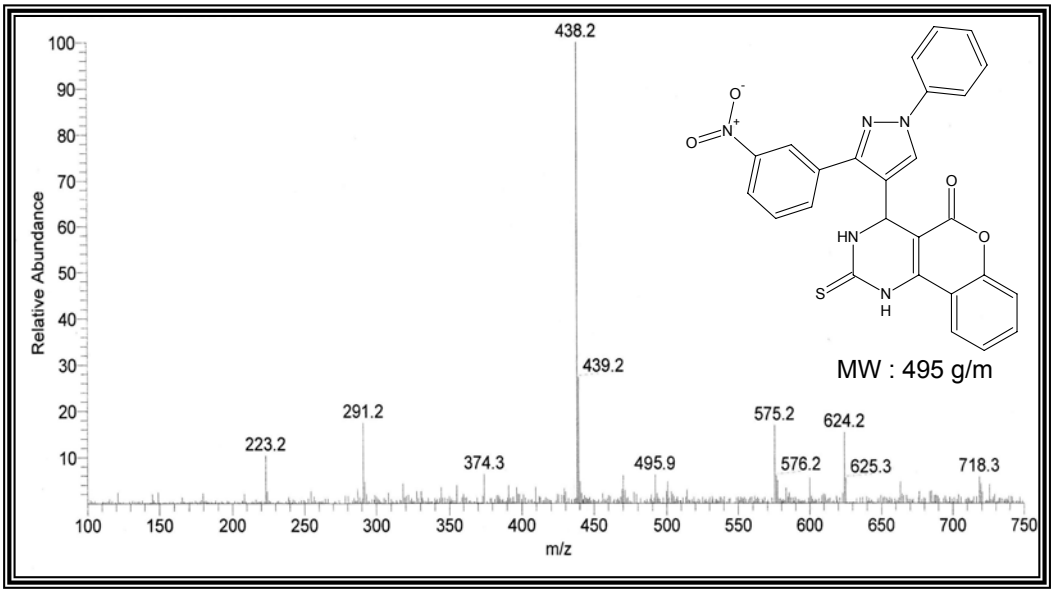


4.15.12 ^{1}H NMR Spectrums of DNJ-1603

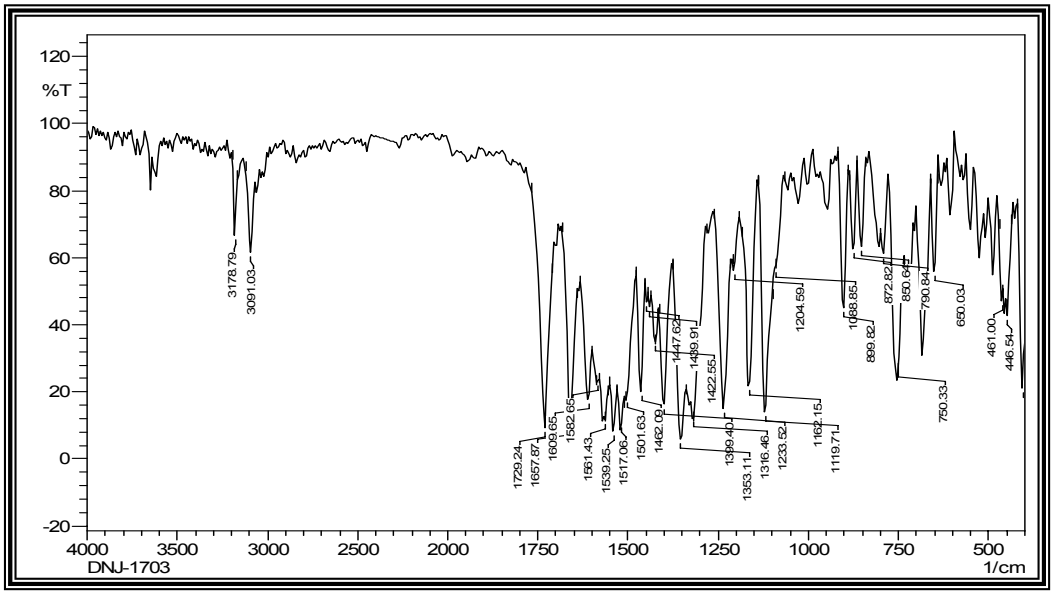




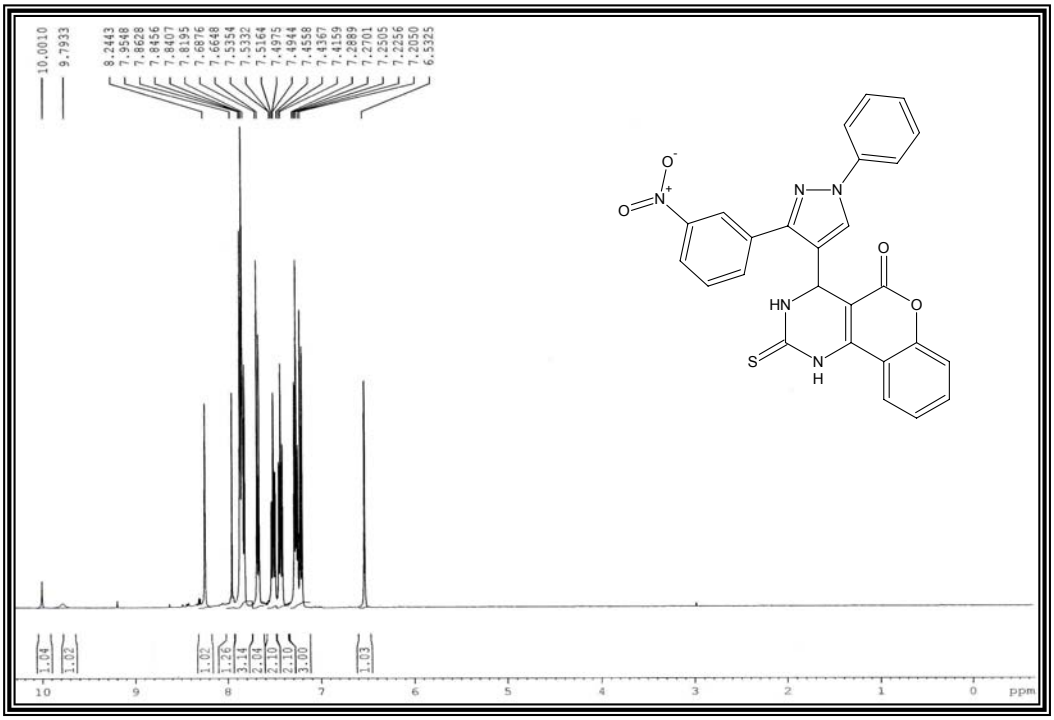
4.15.13 Mass Spectrum of DNJ-1703

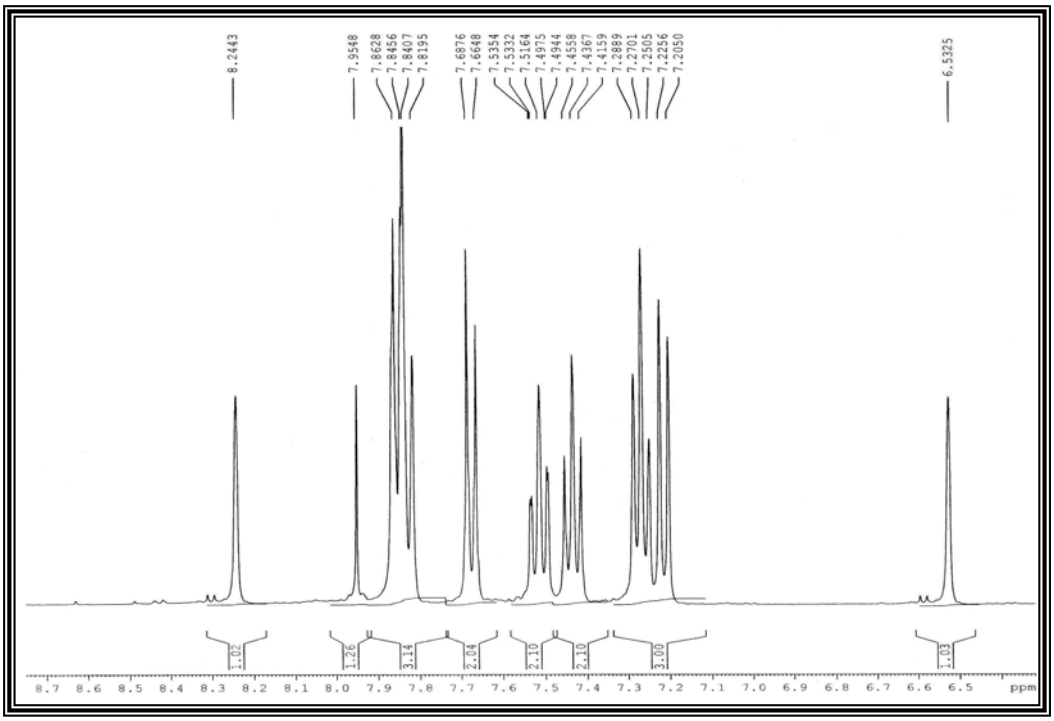


4.15.14 IR Spectrum of DNJ-1703



4.15.15 ^{1}H NMR Spectrums of DNJ-1703





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CHAPTER – 5

SYNTHESIS AND CHARACTERIZATION OF SOME NOVEL MANNICH BASES OF ARYL AMINO COUMARINS

5.1 INTRODUCTION TO ARYLAMINOCOUMARIN

Many coumarin molecules are known in literature but 4-aminocoumarin is not much reported. The preparation of 4-aminocoumarin and other 4-arylaminocoumarins are pioneered by various research workers.

Apparently it is found that 4-aminocourmarin is prepared by direct method by removing acidic hydroxyl group (1) with amino group (3) in one step only, but alternate route is to convert the hydroxyl group (1) into chloro group (2) and then convert it into amino group (3) by appropriate reagent for substitution. (Fig. 5.1)

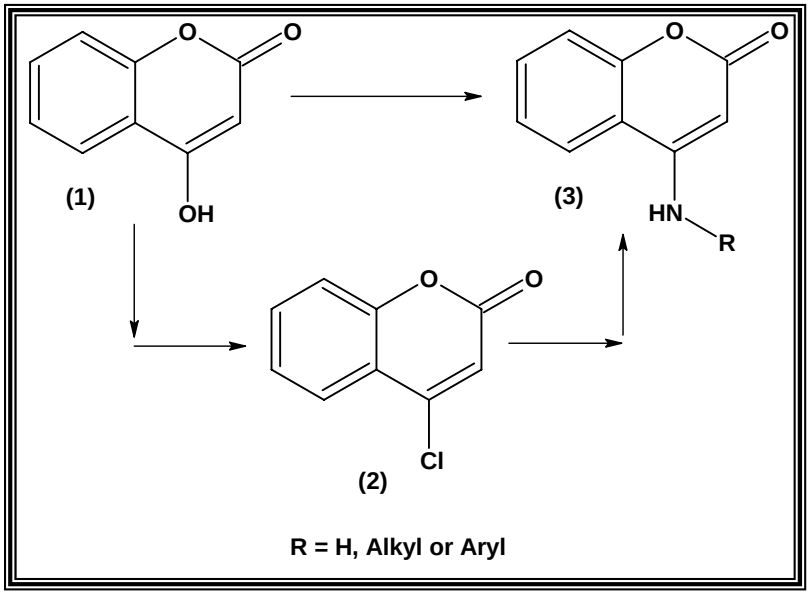


Fig. 5.1

The conversion of 4-hydroxy coumarin can also be afforded by a direct one step method using appropriate arylamine using solvents, without solvents under conventional heating or by using microwave assisted synthetic strategy.

A small review of current update is included.

Anschutz [1] reported the synthesis of 4-anilinocoumarin during his pioneering work by heating 4-hydroxycoumarin with aniline.

Checchi and Vettori [2] prepared 4-aminocoumarin-3-sulphonamide and its derivatives. Sulphonation of 4-hydroxycoumarin in absence of any solvent, with an excess of chlorosulphonic acid yielded 3-sulphonic acid, which was converted to its potassium salt and on further chlorination with phosphorous oxychloride, afforded 4-chlorocoumarin sulphochloride. The treatment of either ammonia or primary aliphatic and aromatic amines led to the formation of 4-aminocoumarin-3-sulphonamide and its derivatives.

Zagorevskii [3] reported that the action of liquor ammonia on 4-chlorocoumarin in the presence of copper powder exclusively afforded the 4-aminocoumarin. In another method, 4-chlorocoumarin when treated with concentrated ammonium hydroxide in dioxane for 40 hours at room temperature afforded 4-aminocoumarin in 25% yield and o-hydroxyphenylpropionamide (52% yield) [4, 5] due to opening of the lactone ring. However, only the desired 4-aminocoumarins were obtained in some cases. [6-9] (Fig. 5.2)

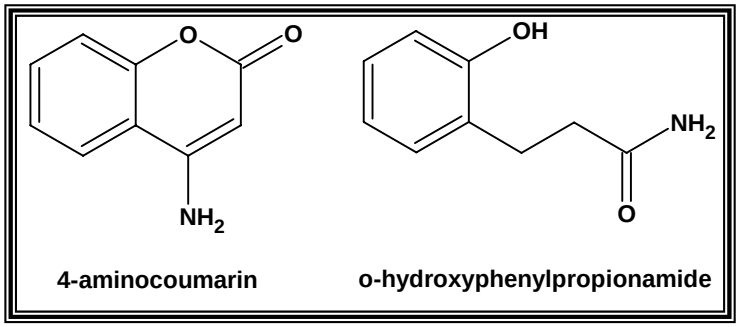


Fig. 5.2

Mustafa *et. al.* [10] reported the synthesis for the preparation of 4-anilinocoumarin in low yield by refluxing 4-hydroxycoumarin with aniline in ethanol.

Wolfbeis [11] reported the synthesis of 4-arylaminocoumarin from 4-hydroxycoumarin by direct condensation with anilines.

4-arylaminocoumarins were prepared by treatment of an ethanolic solution of 4-hydroxycoumarin and o-aminobenzaldehyde under reflux condition, which on further cyclization afforded 6*H*-[1] benzopyrano [4, 3-b] quinoline. [12] This has proved the way for another clean method for the synthesis of 3, 4-fused systems on coumarin nucleus. (Fig. 5.3)

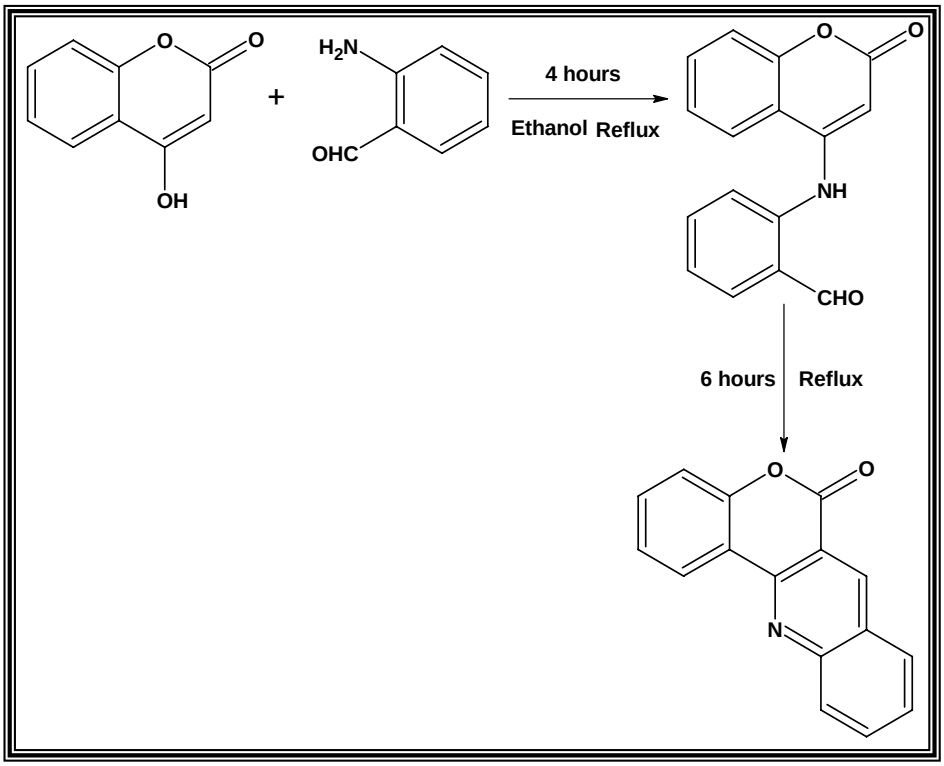


Fig. 5.3

Asherson *et. al.* [13] heated dicoumarol and 4-hydroxycoumarin with aniline to yield 4-arylaminocoumarin. Which was further validated by Conlin *et. al.* [14] while they heated dicoumarol and 4-hydroxycoumarin with aniline, benzylamine and cyclohexylamine under reflux to yield corresponding anil of 4-hydroxycoumarin.

Stunic *et. al.* [15] synthesized *N*-substituted-(3-nitrocoumarinyl) aminoacids from 4-chloro-3-nitrocoumarin in good yield. (Fig. 5.4)

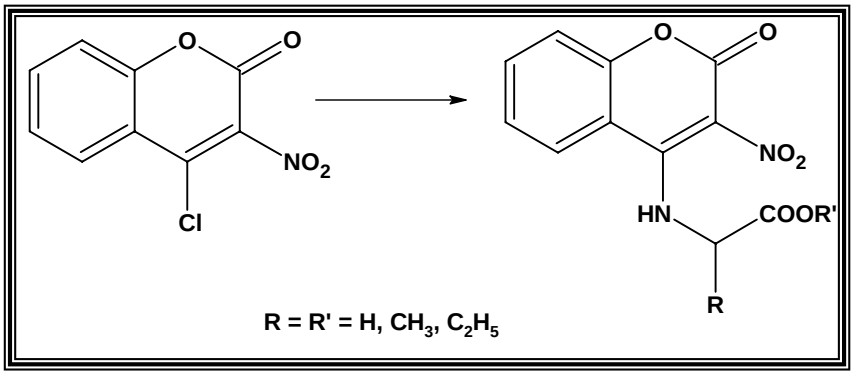


Fig. 5.4

Bhatt and Thakor [16] prepared 4-anilinocoumarins by direct condensation of 4-hydroxycoumarins with different amines, thus opening the single step route of such arylaminocoumarin derivatives.

Joshi [17] and Berghaus [18] have also independently reported the synthesis of 4-amino [1] benzopyrans as intermediate products during annelation.

Reddy *et. al.* [19] carried out condensation reaction of 4-hydroxycoumarin and 2-aminothiophenol in dimethylsulfoxide. This cyclized product was desulfurized with Raney-Nickel to give 4-arylaminocoumarin.

Bardan *et. al.* [20] have prepared 4-aminocoumarin by condensation of primary and secondary amine with 4-chlorocoumarin under reflux in xylene.

In another different approach, during the study of β-ketoenamino functionality, the 4-hydroxycoumarins were directly converted to 4-amino / 4-arylamino coumarins by Ivanov *et. al.* [21]

Kirpichenok *et. al.* [22] reacted 3-iodo-7(dialkylamino) coumarins with different secondary amines (*viz.* diethylamine, piperidine, morpholine, imidazole and benzimidazole) in order to get 4-(substituted amino) coumarin derivatives in good yield.

Tabaković *et. al.* [23] also reported the preparation of 3-nitro-4-anilinocoumarin by refluxing 3-nitro-4-coumarinyl-*N*-phenyldithiocarbamate in dimethylformamide for 30 minutes.

Hamdi *et. al.* [24] reported that heating 1, 2-phenylenediamine with the 4-hydroxycoumarin in ethanol, two products were obtained, one was *N*-(2-aminophenyl)-3-hydroxy-3-(2-hydroxyphenyl) acrylamide and another was 4-[(2-aminophenyl) amino]-2*H*-chromen-2-one. While 1, 4-phenylenediamine was refluxed in xylene with 4-hydroxycoumarin, it also gave two products, one was 4-[(4-aminophenyl) amino]-2*H*-chromen-2-one and another was the dimer.

Papousis *et. al.* [25] prepared 3-phenyliodonium tosylate from 4-aminocoumarin using [hydroxyl(tosyloxy)iodo]benzene which upon basification converted to its conjugated acid which on refluxing in acetonitrile gave 3-iodo-4-anilinocoumarin. Further they reported that treating 3-iodo-4-anilinocoumarin with $PdCl_2(PPh_3)_2$ in triethylamine and tetrahydrofuran gave 4-anilinocoumarin.

Hamdi *et. al.* [26] further tried to explain different possibilities on reaction of 4-hydroxycoumarin with 1, 2-diamino ethane in isopropyl alchohol for 4 hours. (Fig. 5.5)

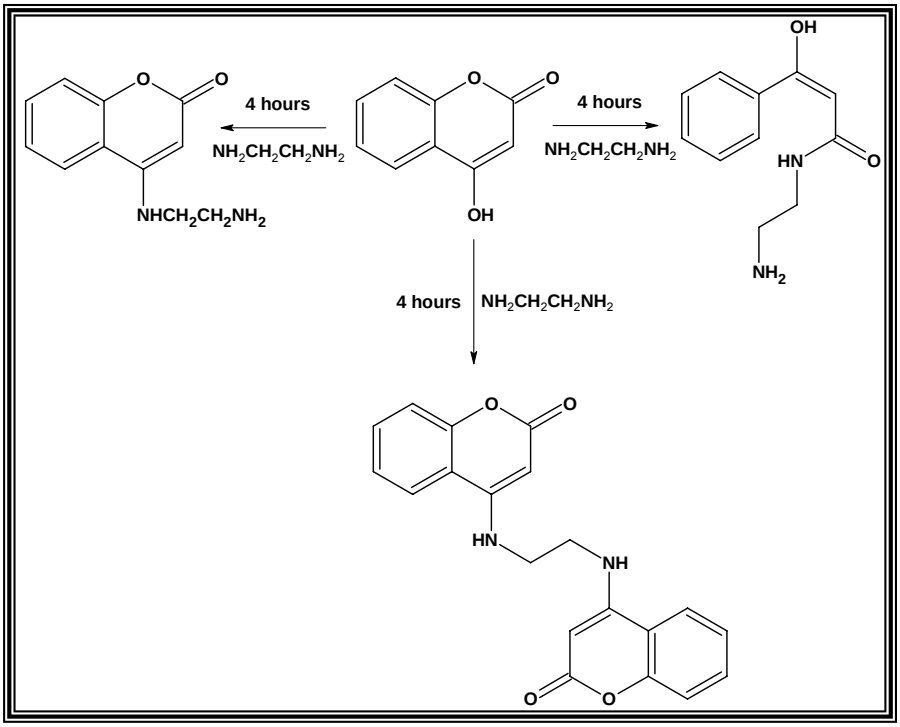


Fig. 5.5

Andrea *et. al.* [27] synthesized 4-(2'-Aminophenyl) amino-3-coumarincarbaldehyde by condensing 4-chloro-3-coumarin carbaldehyde with o-phenylene diamine in presence of triethylamine in refluxing ethanol.

Kováč *et. al.* [28] synthesized substituted 4-(substituted amino)coumarin by reacting coumarin-4-sulphonamide with phenoxy *N*-(substituted)carbamate in presence of DBU (diazabicycloundecene) under reflux, while *N*-(4-isopropylphenyl)-4-aminocoumarin was synthesized by reacting coumarin-4-sulphonamide with 4-isopropylphenyl isocyanate in presence of DBN (diazabicyclononene) under reflux in dioxane.

Stoyanov *et. al.* [29] reported that the reaction of 4-hydroxycoumarin with some primary amines and morpholine under microwave irradiation occurred without opening of the lactone ring to give *N*-substituted 4-aminocoumarins in excellent yields. Under the same experimental conditions, 4-hydroxy-6-methyl-2-pyrone reacted with benzylamine or 2-phenylethylamine to give the corresponding *N*, *N'*-di substituted 4-amino-6-methyl-2-pyridones. The main advantages of this procedure are dramatically shortened reaction time, higher amine utilization and considerably improved yields.

Soman [30] prepared 4-arylamino derivative of 4-hydroxy-11-methyl-2*H*-[1] benzofuro [3, 2-g] chromen-2-one by refluxing them in dimethylformamide for shorter time period.

Chavan [31] prepared 4-aryl- and 4-alkylaminocoumarins by reaction of 4-hydroxycoumarin with amines under microwave irradiation using solvent-free conditions in good to excellent yields.

Shcherbakov *et. al.* [32, 33] reported that the 4-hydroxy-5, 6, 7, 8-tetrafluorocoumarin reacts with the monoamines to form salts under the mild conditions or the 4-alkyl (aryl) aminocoumarins on refluxing in o-xylene. The 3 – acetyl - 4- hydroxyl - 5, 6, 7, 8 - tetrafluorocoumarin reacts with the strong basic amines in the polar solvents to give salts that can be transformed into the 3 – alkylaminoethylidene - 5, 6, 7, 8 – tetrafluorobenzopyran - 2, 4 - diones. The later can be obtained by reaction of the 3 - acetylcoumarin with

different amines. By reacting 3 - acetylcoumarin with the strong basic amines in dimethylsulfoxide gave 7 – alkylamino – 3 – alkylaminoethylidene - 5, 6, 8 - trifluorobenzopyrandiones. The 3 – acetimidoyl – 4 – hydroxyl - 5, 6, 7, 8 - tetrafluorocoumarin affords the 3 - alkylaminoethylidenebenzopyrandiones with the monoamines, but in dimethylsulfoxide the 7-substituted 3-acetimidoyl-5, 6, 8-trifluorobenzopyrandiones or the 7-alkylamino-3-alkylaminoethylidene-5, 6, 8-triflurobenzopyrandiones can be obtained.

Ghosh *et. al.* [34] converted 2-Aminochromones into 4-chlorocoumarins by reacting them with phosphorous oxychloride. Which on subsequent treatment with ethylenediamine gave 2, 3, 4, 5-tetrahydro-7-(2'-hydroxyphenyl)-1, 4-diazepin-5-ones and 4-[(2-aminoethyl) amino] coumarins in ethanol. While in dimethylformamide, it gave [4-(2-formamidoethyl) aminocoumarins].

Ganina *et. al.* [35] synthesized different 4-arylaminocoumarins in high yields by reacting 4-hydroxycoumarin trifluoromethanesulfonates with different amines in refluxing dioxane with the presence of pyridine.

Strakova *et. al.* [36] heated 4-hydroxycoumarin with aniline, p-toluidine, p-anisidine and benzylamine without solvent at higher temperature to yield the corresponding 4-arylaminocoumarins. These compounds were converted to *N*-substituted 4-amino-3-(chloroacetyl) coumarins by treating them with excess of chloroacetyl chloride. The later products while reacted with thiourea gave 3-(2-amino-4-thiazolyl)-4-aminocoumarins.

Zhang *et. al.* [37] generated 4-amino-3-arylcoumarin by consecutive Suzuki-Miyaura reaction / nucleophilic substitution of 3-aryl-4-tosyloxy coumarin using potassium carbonate and ethanol.

5.2 BIOLOGICAL ACTIVITIES ASSOCIATED WITH 4-ARYLAMINOCOUMARINS AND ITS DERIVATIVES

Spalding *et. al.* [4] studied 4-morpholino coumarins as analogue of the camaqauin (quinoline-antimalarial) for antimalarial activity in the plasmodium gallinaceum infection in chicks, but the compounds were found to be inactive, while 7-(4-aminosulphonamide) coumarins were found to possess stronger tubercular activity. [38]

Bechmann [39] studied the antibacterial activity of the compounds for the type shown in Fig. 5.6. 3-nitro-4-(2-pyridylamino) coumarins were tested for their antimicrobial activity against *S. aureus*, *E. coli* and *C. alebicans*. [40]

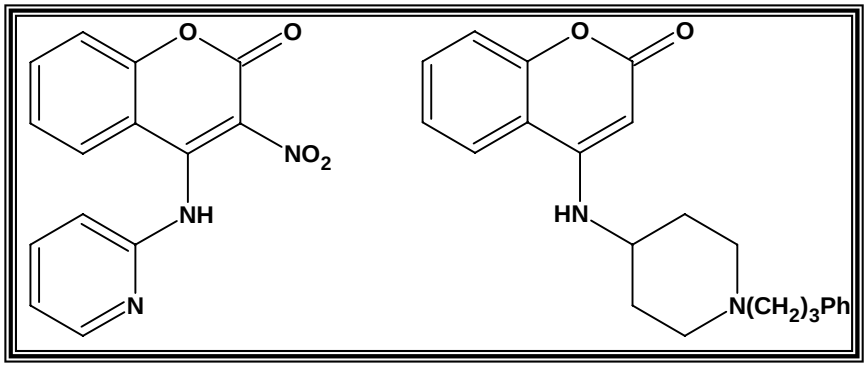


Fig. 5.6

Leurquin [41] reported orally active 4-piperazinylcoumarin as psychotropic compounds.

Zagorevskii *et. al.* [42, 43] have prepared several 3-nitro-4-aminocoumarins possessing neutropic activity, generally inhibiting spontaneous locomotor activity and decreasing hypersensitivity induced by phenamine and prolonging sleep due to a barbiturate drug thiopental. Zagorevskii [44] further reported weak psychotropic activity in arylamino coumarin derivatives of the type shown in Fig. 5.7.

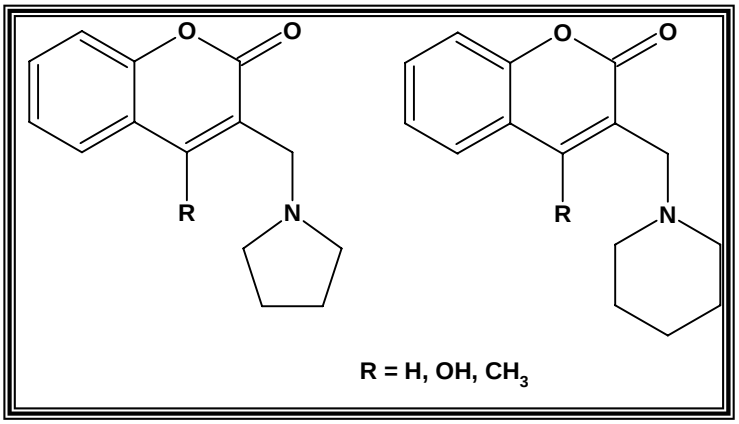


Fig. 5.7

Bardan *et. al.* [45] reported the synthesis of a novel series of substituted-aminocoumarins (1) (R, R^1 = H, OH, *N*-arylamino) and the preliminary evaluation of their *in vitro* antimicrobial activity was described. The reaction of (1) (R = OH; R^1 = H) (Fig. 5.8) with a numbers of primary or cyclic secondary amines afforded the corresponding aminocoumarin derivatives. Antimicrobial screening showed that some of these novel compounds possess moderate activity against *Bacillus subtilis* and *Bacillus pumilus*.

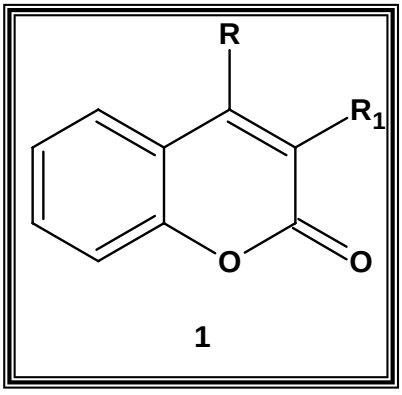


Fig. 5.8

In last few years, new structural classes of tyrosine kinase inhibitors (anticancer agents) have begun to emerge which exhibit enormous improvements in potency and specificity over prior compounds. Most of these compounds are directed against either the epidermal growth factor (EGF) or platelers derived growth factor (PDGF) receptor tyrosine kinease and have

capacity to effectively suppress their targets in cells. Structures types shown in Fig. 5.9 were found to be most effective. [46, 47]

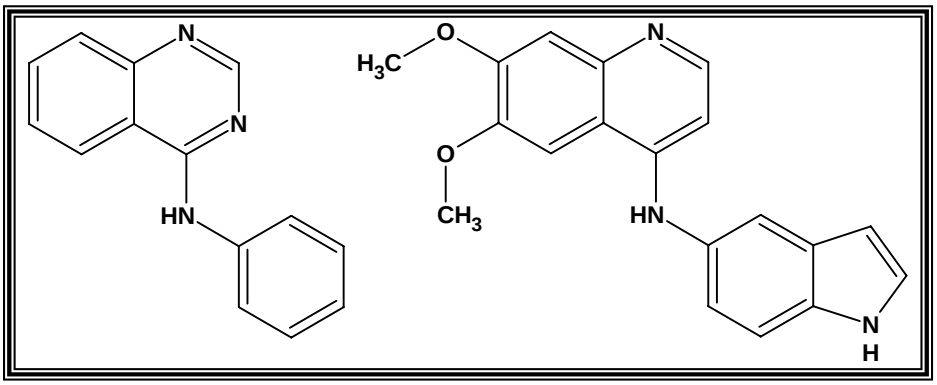


Fig. 5.9

As structural features of above compounds tallies with the title compounds (i.e. 4-aryl aminocoumarins), it induced to study the possible alteration in the activity by substituting the benzenoid part either with electrone withdrawing group (EWG) or electrone donating group (EDG) and also by shifting of the heterocyclic system for 4-anilinoquinazoline to other structures like 4-anilinocoumarins.

Shan and coworkers [48-53] have synthesized the following type of 4-arylaminocoumarins shown in Fig. 5.10 and screened for anti-HIV activity against HIV-1 (strain HTLV-III_B / LAI) [54] and HIV-2 (strain LAV-2_{ROD}) [55] and antitubercular activity against *M. tuberculosis* $H_{37}Rv$.

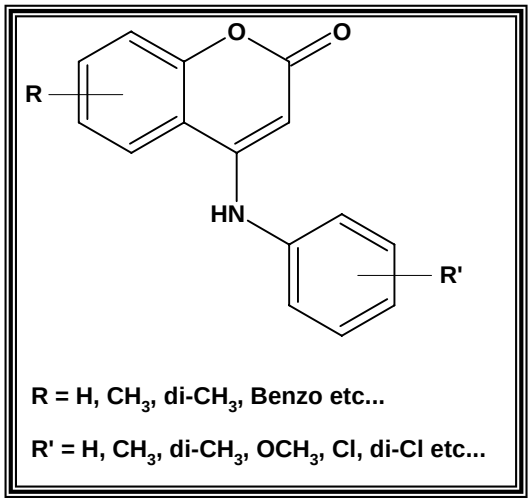


Fig. 5.10

Recently Hamdi *et. al.* [56] reported the reaction of 4-hydroxycoumarin in toluene with a variety of aromatic bis (nucleophilic) compounds, where 3-(dimethylaminomethylene)-2, 4-chromandione was used as a key intermediate for the preparation of bis [*N*-(4-oxocoumarinylmethylene)]-1, 4-diamines. Alternative synthetic procedures and antibacterial activity data of some of the new compounds were given. Compounds thus prepared, i.e., 1, 3-dihydro-4-(2-hydroxyphenyl)-2*H*-1, 5-benzodiazepin-2-one, 4-[(2-mercaptophenyl) amino]-2*H*-1-benzopyran-2-one and 4-[(2-hydroxyphenyl) amino]-2*H*-1-benzopyran-2-one were evaluated for their antibacterial activity against *Staphylococcus aureus*.

Braccio *et. al.* [57] obtained the *N*-substituted tricyclic 2-aminochromone derivatives by treating the corresponding (methylthio) or (methylsulfinyl) derivatives with an excess of the properly substituted amines. Compound (1) (R = R_1 = Ph) was synthesized through the reaction of β-naphthol with the base / $POCl_3$ reagent. The *N*-substituted 4-aminocoumarin bicyclic and tricyclic derivatives such as (2) [X = OCH_3, Y = H (3)] were prepared by treating the corresponding chloro derivatives with the excess of properly substituted amines. Compounds were tested *in vitro* for their antiproliferative activity (DNA synthesis inhibition in Ehrlich cells) and cytotoxicity (MTT test in HeLa cells). The inhibitory properties of three selected compounds (3), (2) (X = Y = OCH_2O) and (4) on protein and RNA syntheses in Ehrlich cells were also evaluated. Among the twenty seven compounds tested, ten 4-aminocoumarin derivatives and two 2-aminochromone derivatives, (4) and (1) (R = H, R_1 = 4-chlorophenyl) showed an appreciable antiproliferative activity (IC_{50} range: 1.74-13.8 μM), whereas only four compounds exhibited a comparable cytotoxic activity (IC_{50} range: 4.95-12.9 μM). (Fig. 5.11)

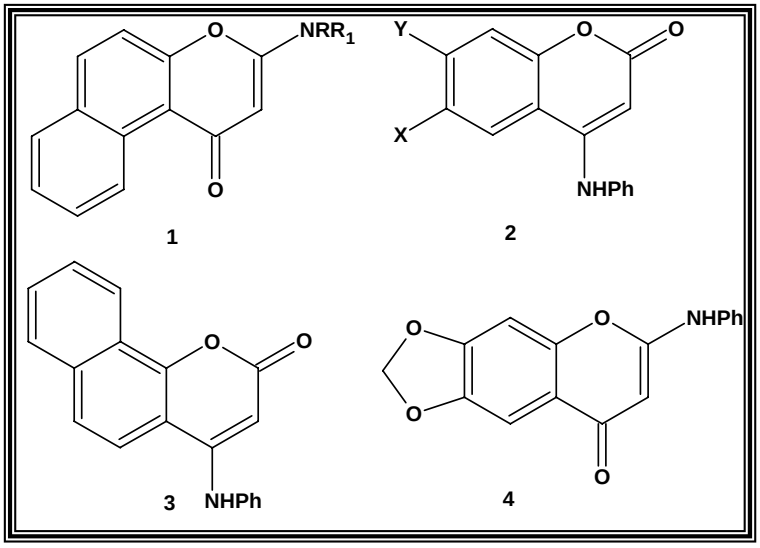


Fig. 5.11

Chavda *et. al.* [58] replaced the phenolic hydroxyl group of 4-hydroxycoumarins by different amines in a single step method by direct heating to get newly substituted 4-amino derivatives which were screened for their antimicrobial activity.

5.3 MANNICH REACTION ON 4-HYDROXYCOUMARIN

Mannich reaction has been reported [20, 46, 59, 60, 61] on 4-hydroxycoumarin where 3-substituted *C*-Mannich bases of 4-hydroxycoumarin have been obtained using different secondary amines and formaldehyde. Where these Mannich bases are associated with antimicrobial activity [46] and psychotropic activity. [20]

5.4 *C*-MANNICH BASES OF ARYLAMINOCOUMARINS

Literature survey revealed that Mannich reaction has not been carried out on 4-arylaminocoumarins yet, but similar kinds of compounds have been prepared by Savel'ev *et. al.* [60] Where, firstly they carried out Mannich reaction on 4-hydroxycoumarin to give 3-substituted-4-hydroxycoumarin (i. e. *C*-Mannich bases) which consequently converted into 3-substituted-4-chloro

coumarin and treatment of 4-chloro derivative with primary and secondary amines gave title compounds with weak psychotropic activity. (Fig. 5.12)

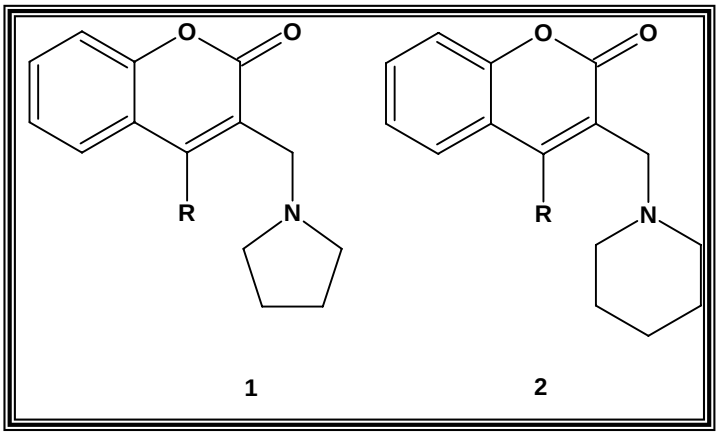


Fig. 5.12

Where R = MeNH, BuNH, $PhCH_2NH$, PhNH, Me_2N, Et_2N, pyrrolidino, piperidino, morpholino, 4-methylpiperazino.

5.5 AIM OF CURRENT WORK

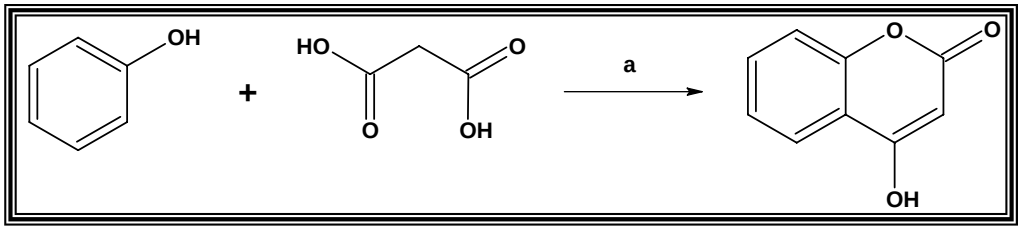
Our group is involved in the synthesis of aryl amino coumarins since last few years, where, aryl amino coumarins showed good antitubercular activity. Previously, preparation of differently substituted aryl amino coumarins was only the aim. Afterwards, the aryl amino coumarins were further cyclized to give fused pyridine as well as fused benzothiazine derivatives, which showed good antitubercular as well as anticancer activity. Looking to the interesting biological profile showed by coumarin benzothiazine derivatives and their unexplored chemistry part and from the literature survey, it was decided to modify previously prepared fused benzothiazines derived from aryl amino coumarins into *N*-substituted benzothiazine derivatives so that *N*-alkylation was carried out on coumarin benzothiazine derivatives by different methods but it failed. Then *N*-alkylation was carried out on aryl amino coumarin derivatives but it also failed. Afterwards, new route was thought by which benzothiazine derivatives could be synthesized that is why the Mannich reaction was carried out on aryl amino coumarins in order to prepare *N*-Mannich bases of aryl amino coumarins and their further cyclization into benzothiazine derivatives. But to our surprise, the Mannich reaction on aryl amino coumarin resulted into unusual new product formation which has been discussed in results and discussion in detail.

Literature revealed that Mannich reaction is not reported on aryl amino coumarins but similar kind of scaffold is reported [60]. Where Mannich reaction was carried out on 4-hydroxy coumarin and the hydroxyl group present at 4th position in coumarin nucleus was converted into chloro which was easily removed by the treatment of aromatic primary amine. As discussed in chapter – 1, Mannich bases can be synthesized by Mannich reaction on nitrogen of secondary amine by using simplified methodology and easy work up and this inspired us to develop some new aryl amino coumarin derivatives by Mannich reaction for our targeted work. Formaldehyde solution (37-41 %) and different type of secondary amines were used to acquire Mannich bases under acidic

conditions. Thus the chemistry of Mannich reaction on arylaminocoumarins is not only interesting but also novel and is reported here for the first time.

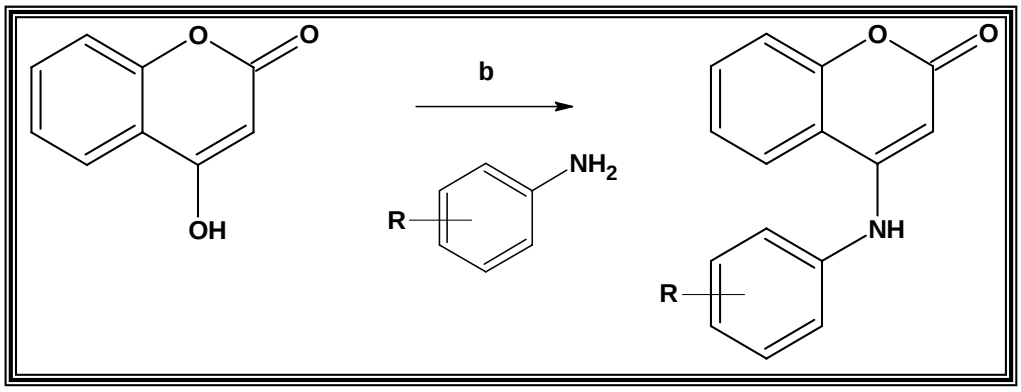
5.6 REACTION SCHEMES

5.6.1 PREPARATION OF 4-HYDROXY COUMARIN



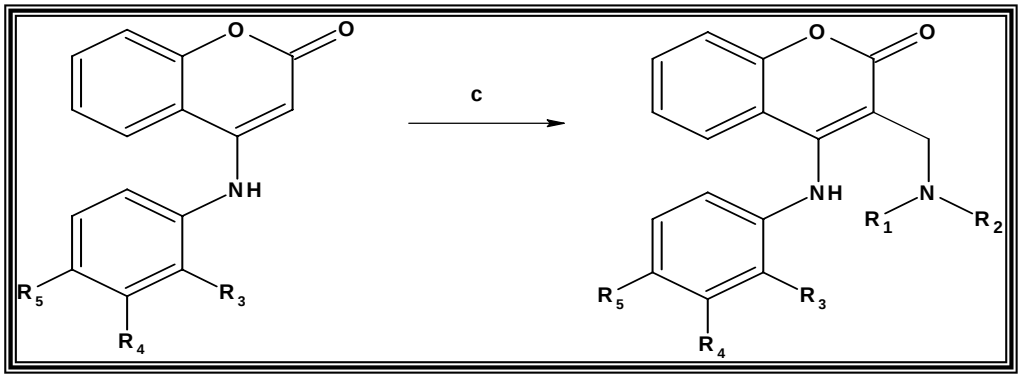
Reagents / Reaction Condition (a): Anhydrous $ZnCl_2$, $POCl_3$ / 70°C, 12 hours.

5.6.2 PREPARATION OF 4-(SUBSTITUTED ANILINO) COUMARIN



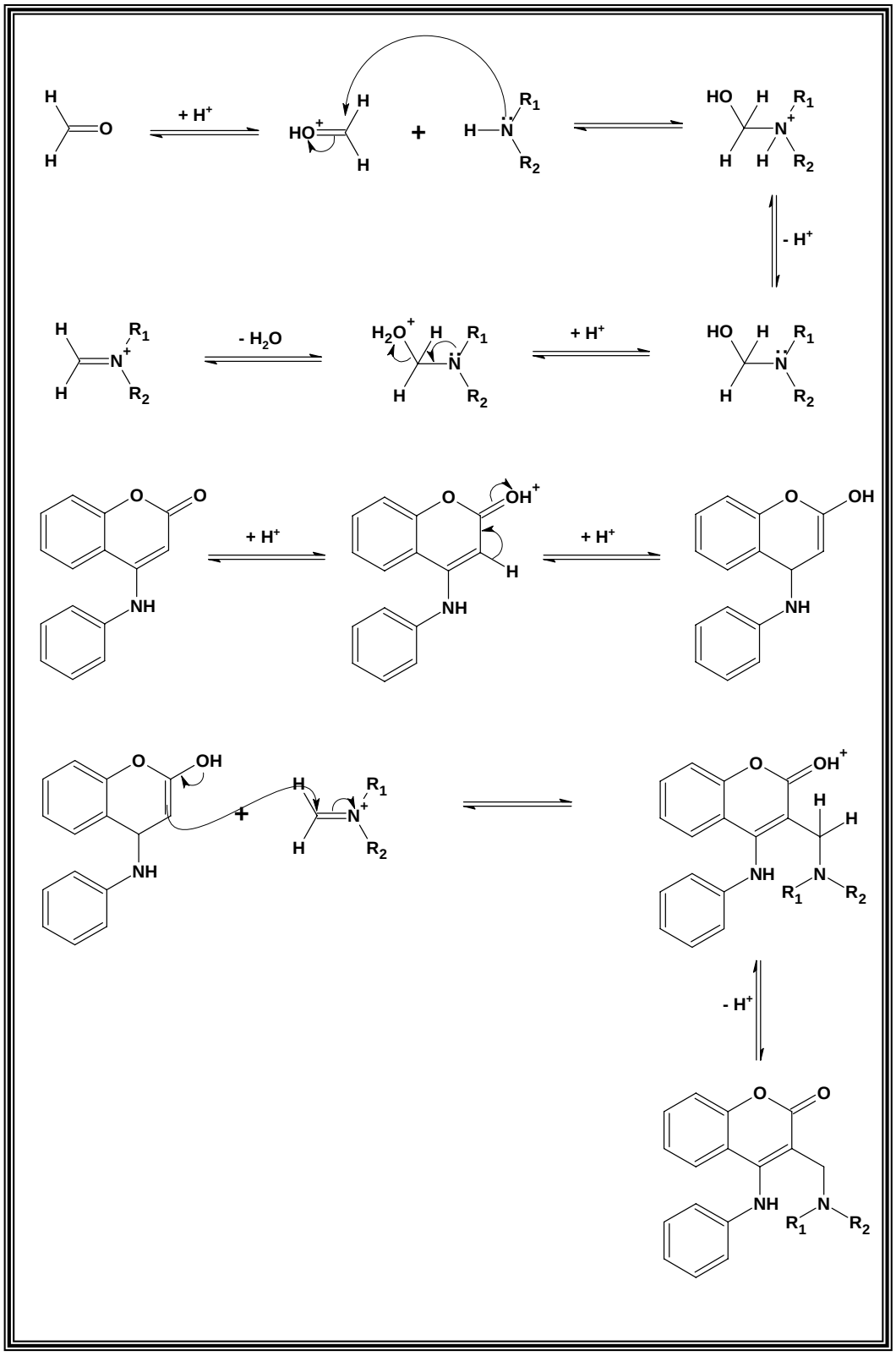
Reagents / Reaction Condition (b): Substituted primary amines / MW, 320 Watt.

5.6.3 PREPARATION OF ARYL AMINO COUMARIN *C*-MANNICH BASES



Reagents / Reaction Condition (c): Formaldehyde (37-41 % solution), Secondary amine, DMF, Con. HCl / Warm→Room Temperature

5.7 PLAUSIBLE REACTION MECHANISM



5.8 EXPERIMENTAL

5.8.1 PREPARATION OF 4-HYDROXY COUMARIN

It was prepared according to the method reported by Shah *et. al.* [a] Yield - 55 %, MP - 210-212°C (210-212°C [a]).

5.8.2 GENERAL PROCEDURE FOR THE PREPARATION OF 4-(SUBSTITUTED ANILINO) COUMARIN

Mixture of 0.1 mole of 4-hydroxy coumarin and 0.1 mole of appropriately substituted primary amine was irradiated neat under microwave irradiation using domestic microwave oven (LG MS-192 W) for desired time at 320 Watt. The reaction mixture was cooled to room temperature and methanol was added to it. The separated solid was filtered and washed with methanol. It was dried and recrystallized with dimethylformamide to give 4-(substituted anilino) coumains. Required reaction time and obtained results are summarized as under.

Code No.	Reaction Time (min.)	% yield	Code No.	Reaction Time (min.)	% yield
	320 Watt			320 Watt	
AAC-1	6.0	81	AAC-8	6.4	91
AAC-2	4.1	85	AAC-9	5.3	83
AAC-3	6.3	79	AAC-10	7.3	80
AAC-4	5.2	88	AAC-11	7.1	79
AAC-5	5.5	84	AAC-12	7.0	88
AAC-6	4.3	90	AAC-13	5.2	84
AAC-7	6.2	77			

[a] A. K. Shah, N. S. Bhatt and V. M. Thakor; *Curr. Sci.*, **1984**, *53(24)*, 1289.

5.8.3 GENERAL PROCEDURE FOR THE PREPARATION OF DNJ-1001 TO DNJ-1015

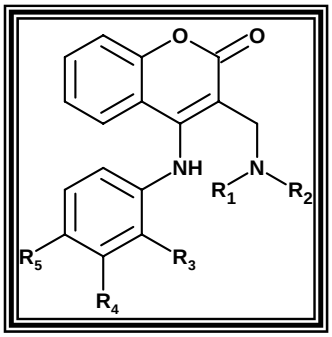
0.01 mole of appropriately substituted aryl amino coumarin was dissolved into 30 ml of dimethylformamide. 1 ml of formaldehyde (37-41 % solution) and 0.01 mole of an appropriate secondary amine were added into above reaction mixture under acidic condition. The content was heated on boiling water bath for some time and the reaction mixture was left overnight at room temperature to furnish the product. The separated crystals were filtered off and it was washed with acetone to give white to pale yellow coloured shining crystals. The purity of the compound was checked by silica gel-G F_{254} thin layer chromatography using toluene : ethyl acetate (7 : 3) as a mobile phase. Recrystallization was not required in any case though it was carried out from acetone / methanol. (Physical data of the synthesized end products are summarized in the table 5.9.1)

1-methyl piperazine was used in case of DNJ-1001 while 1-benzyl piperazine was used in case of DNJ-1003. Rests of the compounds were synthesized using 1-ethyl piperazine.

Moreover single crystal of DNJ-1003 was developed in order to prove the X-ray crystallographic structure. Acquired crystal structure and related data are also given.

5.9 PHYSICAL DATA TABLES

5.9.1 Physical data of 4-(substituted aniline) coumarins *C*-Mannich bases (DNJ-1001 to DNJ-1015)



Code	Substitution		MF	MW (g/m)	MP (°C)	R_f	% Yield
	R_1 & R_2	R_3, R_4 & R_5					
DNJ 1001	1-methyl piperazinyl	Anilino	$C_{21}H_{23}N_3O_2$	349	158	0.40	70
DNJ 1002	1-ethyl piperazinyl	Anilino	$C_{22}H_{25}N_3O_2$	363	164	0.43	65
DNJ 1003	1-benzyl piperazinyl	Anilino	$C_{27}H_{27}N_3O_2$	425	176	0.50	63
DNJ 1004	1-ethyl piperazinyl	2-chloro phenyl amino	$C_{22}H_{24}ClN_3O_2$	397	170	0.44	59
DNJ 1005	1-ethyl piperazinyl	3-chloro phenyl amino	$C_{22}H_{24}ClN_3O_2$	397	168	0.46	61
DNJ 1006	1-ethyl piperazinyl	4-chloro phenyl amino	$C_{22}H_{24}ClN_3O_2$	397	162	0.48	55
DNJ 1007	1-ethyl piperazinyl	2-fluoro phenyl amino	$C_{22}H_{24}FN_3O_2$	381	184	0.42	46
DNJ 1008	1-ethyl piperazinyl	4-fluoro phenyl amino	$C_{22}H_{24}FN_3O_2$	381	190	0.44	58
DNJ 1009	1-ethyl piperazinyl	2-methyl phenyl amino	$C_{23}H_{27}N_3O_2$	377	180	0.44	67
DNJ 1010	1-ethyl piperazinyl	3-methyl phenyl amino	$C_{23}H_{27}N_3O_2$	377	186	0.46	63
DNJ 1011	1-ethyl piperazinyl	2-methoxy phenyl amino	$C_{23}H_{27}N_3O_3$	393	174	0.45	44

DNJ 1012	1-ethyl piperazinyl	3-methoxy phenyl amino	$C_{23}H_{27}N_3O_3$	393	166	0.47	56
DNJ 1013	1-ethyl piperazinyl	3-trifluoromethyl phenyl amino	$C_{23}H_{24}F_3N_3O_2$	431	182	0.43	66
DNJ 1014	1-ethyl piperazinyl	Octyl amino	$C_{24}H_{37}N_3O_2$	399	200	0.48	40
DNJ 1015	1-ethyl piperazinyl	2-furyl methyl amino	$C_{21}H_{25}N_3O_3$	367	196	0.41	50

R_f value was calculated using solvent system = Toluene : Ethyl Acetate (70% : 30%)

5.10 SPECTRAL DISCUSSION

5.10.1 MASS SPECTRAL STUDY

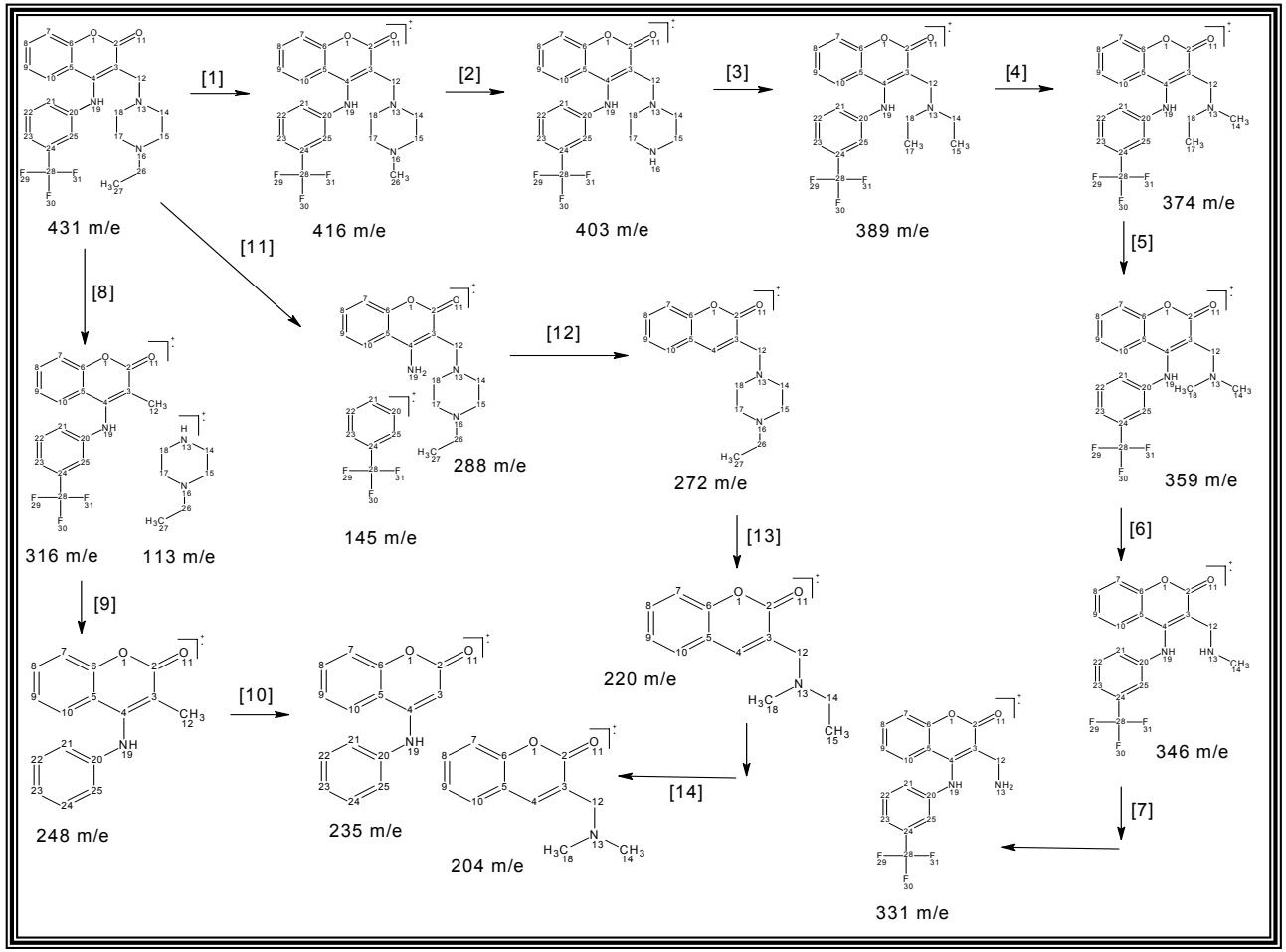
Mass spectra of the synthesized compounds were recorded on **Shimadzu GC-MS QP-2010** model using direct injection probe technique. The molecular ion peak was found in agreement with molecular weight of the respective compound. Characteristic M^{+2} ion peaks with one-third intensity of molecular ion peak were observed in case of compounds having chlorine atom. Fragmentation pattern can be observed to be particular for these compounds and the characteristic peaks obtained for each compound. Probable fragmentation pattern for DNJ-1013 can be discussed as under.

4-[(3-chlorophenyl) amino]-3-[(4-ethylpiperazin-1-yl) methyl]-2*H*-chromen-2-one (DNJ-1013)

1. The target compound showed characteristic molecular ion peak.
2. C_{26}-C_{27} bond cleavage gave characteristic peak at 416 m/e. [1]
3. N_{16}-C_{26} bond cleavage gave characteristic peak at 403 m/e. [2]
4. Cleavage of the bonds C_{15}-N_{16} and C_{17}-N_{15} gave characteristic peak at 389 m/e. [3]
5. Cleavage of the bond C_{17}-C_{18} gave characteristic peak at 374 m/e. [4]
6. C_{14}-C_{15} bond cleavage gave characteristic peak at 359 m/e. [5]
7. Cleavage of the bond N_{13}-C_{18} gave characteristic peak at 346 m/e. [6]
8. N_{13}-C_{14} bond cleavage gave characteristic peak at 331 m/e. [7]
9. Cleavage of the bond C_{12}-N_{13} gave two characteristic peaks. One at 316 m/e and second at 113 m/e. [8]
10. C_{24}-C_{28} bond cleavage gave characteristic peak at 248 m/e. [9]
11. Cleavage of the bond C_3-C_{12} gave characteristic peak at 235 m/e. [10]
12. N_{19}-C_{20} bond cleavage gave two characteristic peaks. One at 287 m/e and second at 145 m/e. [11]
13. Cleavage of the bond C_4-N_{19} gave characteristic peak at 272 m/e. [12]

14. After C_4-N_{19} bond cleavage, cleavage of the bonds C_{17}-C_{18}, C_{15}-N_{16} gave characteristic peak at 217 m/e. [13]
15. C_{14}-C_{15} bond cleavage gave characteristic peak at 204 m/e. [14]

5.10.1.1 FRAGMENTATION PATTERN FOR DNJ-1013



5.10.2 IR SPECTRAL STUDY

IR spectra of the synthesized compounds were recorded on **Shimadzu FT IR 8400** spectrophotometer using Diffused Reflectance Attachment (DRA) System using Potassium Bromide.

All compounds showed the carbonyl stretching frequency near 1700 cm^{-1}. All compounds showed N-H stretching frequency in the region of 3220-3395 cm^{-1}. C-N stretching (2° & 3°) frequency was found in each and every compound. C-H stretching frequencies were observed at 2810-2970 cm^{-1}, while ring skeleton frequencies were observed at 1450-1610 cm^{-1}.

Characteristic frequencies for o, m and p-di substitution were observed in each compound. C-X (X = Cl, F) stretching frequencies were obtained in DNJ-1004, DNJ-1005, DNJ-1006, DNJ-1007, DNJ-1008 and DNJ-1013. All compounds showed C-O-C stretching frequency.

5.10.3 ^{1}H & ^{13}C NMR (APT 25) SPECTRAL STUDY

^{1}H & ^{13}C NMR (APT 25) spectra of the synthesized compounds were recorded on **Bruker Avance II 400** & **Bruker Avance II 300** spectrometer. Sample solutions were made in $CDCl_3$ solvent using tetramethylsilane (TMS) as the internal standard unless otherwise mentioned. Numbers of protons and numbers of carbons identified from H NMR & C NMR spectrum and their chemical shift (δ ppm) were in the agreement of the structure of the molecule. *J* values were calculated to identify o, m and p coupling. In some cases, aromatic protons were obtained as multiplet. ^{1}H & ^{13}C NMR (APT 25) spectral interpretation can be discussed as under.

^{1}H NMR spectral interpretation of 3-[(4-ethylpiperazin-1-yl) methyl]-4-{[3-(trifluoromethyl) phenyl] amino}-2*H*-chromen-2-one (DNJ-1013)

1. One most deshielded proton of secondary nitrogen introduced at fourth position in coumarin nucleus gave singlet at 10.54 δ ppm which is the

most identifiable and characteristic peak for these types of compounds from which it could be prove that the compounds were the *C*-Mannich bases and were not the *N*-Mannich bases.

2. Two protons of methylene group attached at C_3 position of coumarin nucleus gave a sharp singlet in the up-field at 3.76 δ ppm.
3. Three most shielded protons of methyl group of ethyl piperazine ring gave triplet at 1.12 δ ppm.
4. Two protons of methylene group attached on nitrogen atom in piperazine ring and eight protons of piperazine ring merged in the region of 2.15-2.62 δ ppm .
5. Four protons of coumarinyl phenyl ring and four protons of another phenyl ring attached to the secondary nitrogen gave multiplet peaks in the region of 7.00-7.48 δ ppm.
6. *J* values were calculated which were in the agreement of the m substitution.

^{13}C NMR (APT 25) spectral interpretation of 3-[(4-ethylpiperazin-1-yl) methyl]-4-{[3-(trifluoromethyl) phenyl] amino}-2*H*-chromen-2-one (DNJ-1013)

In ^{13}C NMR (APT 25), upward directed peaks were due to C and CH_2 while downward directed peaks were due to CH and CH_3.

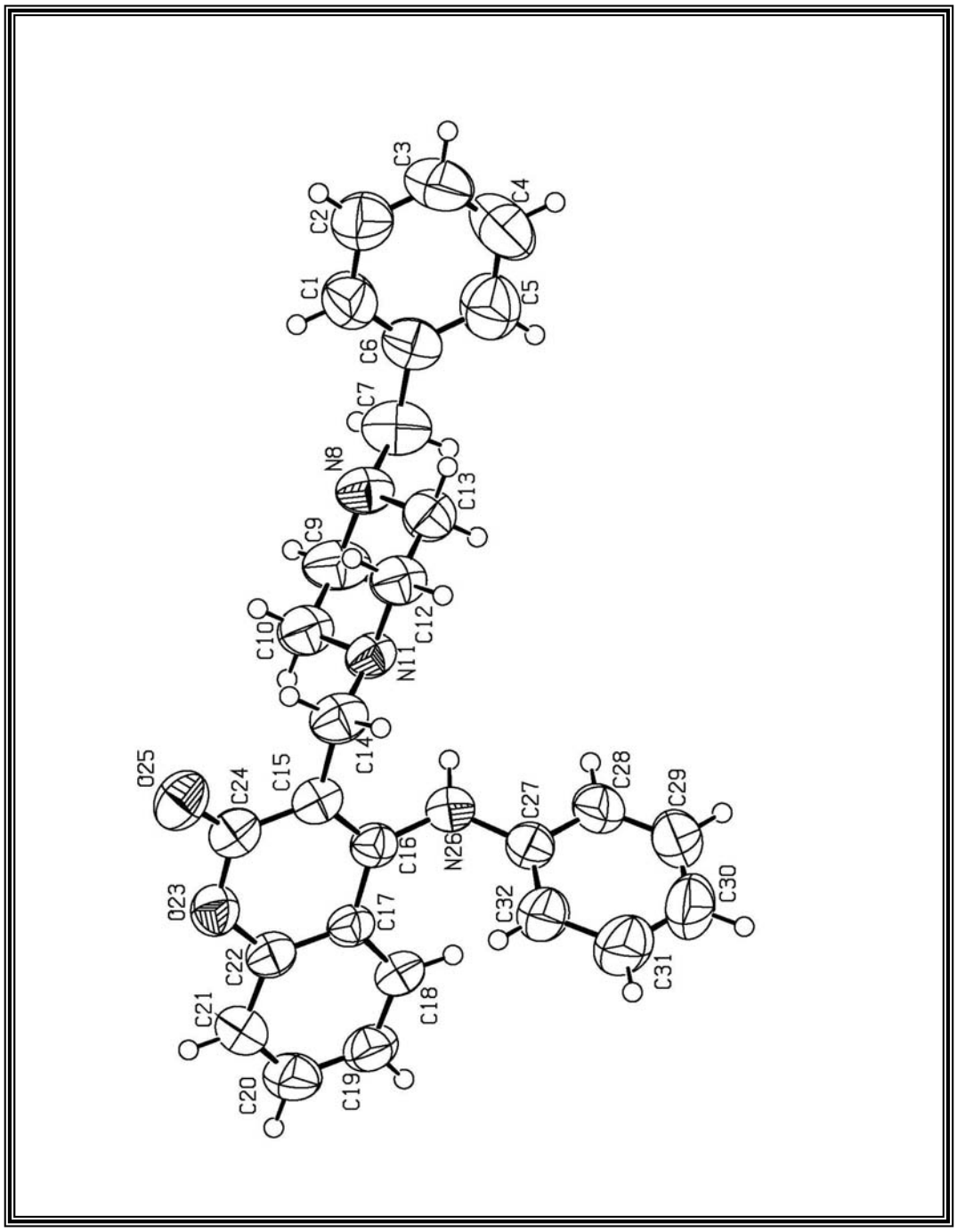
1. Carbonyl group of β keto ester showed upward peak at 162.89 δ ppm.
2. Coumarinyl C_4 carbon showed upward directed peak at 143.29 δ ppm chemical shift value.
3. 132.89 δ ppm chemical shift is due to trifluoromethyl group.
4. C_3 carbon of coumarin nucleus showed peak upward at 105.57 δ ppm from which it could be prove that the compounds were the *C*-Mannich bases and were not the *N*-Mannich bases because if the compounds would be *N*-Mannich bases, they would show peak of C_3 carbon downward due to CH.
5. Peaks at 77.03-77.88 δ ppm are due to the solvent $CDCl_3$.

6. 54.78 δ ppm chemical shift is due to the peak obtained for bridged methylene group (C_{12}) attached between C_3 position and piperazine ring.
7. 53.42 δ ppm showed chemical shift for two piperazinyl methylene carbon atoms C_{15} and C_{17}.
8. 52.72 δ ppm showed chemical shift for two piperazinyl methylene carbon atoms C_{14} and C_{18}.
9. 12.37 δ ppm showed chemical shift for methyl carbon atom C_{27}.
10. Rests of the peaks were due to the aromatic carbon atoms.

5.10.4 ELEMENTAL ANALYSIS

Elemental analysis of the synthesized compounds was carried out on **Vario EL Carlo Erba 1108** which showed calculated and found percentage values of Carbon, Hydrogen and Nitrogen in support of the structure of synthesized compounds. The spectral and elemental analysis data are given for individual compounds.

5.11 X-ray Crystal Structure of DNJ-1003



The compound crystallizes in the Tetragonal crystal class in the space group *I* 41 / *a* with the cell parameters a = 27.7990(6) A°, b = 27.7990(6) A°, c = 12.1800(8) A°, V = 9412.5(7) $A°^3$ for Z=16. The structure reveals that the piperazine ring is in chair conformation. The structure exhibits both inter and intramolecular hydrogen bonds of the type C-H· · ·O and N-H· · ·N.

A study of torsion angles, asymmetric parameters and least squares plane calculations reveals that the piperazine ring in the structure is in a chair conformation with the atoms N_8 and N_{11} deviating 0.2344(26) A° and -0.2380(24) A° from Cermer and Pople plane defined by the atoms $C_9/C_{10}/C_{12}/C_{13}$. This is confirmed by the puckering parameters Q = 0.5786(32) A° θ = 0.36(30)° and Φ = 148.786(5)°. Total puckering amplitude for ten membered ring $O_{17}/C_{16}/C_{15}/C_{24}/C_{23}/C_{22}/C_{21}/C_{20}/C_{19}/C_{18}$ is Q = 0.1063(29) A°. The dihedral angle between the least-squares plane of piperazine ring bridged via -CH_2- to the phenyl ring C_1-C_6 is 83.89(18)° implying that the phenyl ring is nearly perpendicular to the plane of the piperazine ring. Piperazine ring and the coumarin ring bridged via - CH_2- group gives a dihedral angle of 87.67(12), which reflect that the piperazine ring is perpendicular to the plane of the coumarin ring. The dihedral angle between coumarin ring and phenyl ring bridged via NH group is 65.51(11)°, implies that phenyl ring is equatorial to the plane of coumarin ring. The two phenyl rings and the coumarin ring are independently planar. The torsion angle about C_6-C_7-N_8-C_9 being -169.34(27)° and that about C_{15}-C_{14}-C_{12}-C_{13} being 174.02(23)° show that they reflect -anti-periplanar and +anti-periplanar conformation, respectively. At the same time torsion angle about C_{28}-C_{27}-N_{26}-C_{24} is -166.38(26) indicating -anti-periplanar conformation. The structure exhibits both inter and intra molecular hydrogen bonding of the type C-H· · ·O and N-H· · ·N. The intermolecular hydrogen bond C_7-H_7A· · ·O_{17} and C_{10}-$H_{10}A$· · ·O_{25} has a length of 3.290(4) A° and 3.460(4) A° and an angle of 130° and 165°, respectively, with symmetry codes -3/4+y, 5/4-x, -3/4+z and 5/4-y, 3/4+x, -1/4+z respectively. Intramolecular hydrogen bond N_{26}-H_{26}· · ·N_{11}, has a length of 2.861(3) A° and an angle of 119°. The packing of the molecule along c axis is shown in figure.

Table 1: Atomic coordinates and equivalent thermal parameters of the non-hydrogen atoms.

Atom	x	y	z	U_{eq}
C1	0.46520(2)	0.92627(2)	-0.5427(3)	0.1089(1)
C2	0.50219(2)	0.8939(2)	-0.5634(4)	0.1255(2)
C3	0.50455(2)	0.85162(2)	-0.5106(4)	0.1106(1)
C4	0.47037(2)	0.84063(2)	-0.4344(4)	0.1118(1)
C5	0.43328(1)	0.87240(1)	-0.4133(3)	0.0959(1)
C6	0.42957(1)	0.91516(1)	-0.4671(3)	0.0760(9)
C7	0.38768(1)	0.94850(1)	-0.4486(3)	0.0902(1)
N8	0.38247(8)	0.96407(8)	-0.3344(2)	0.0684(7)
C9	0.33616(1)	0.98870(1)	-0.3193(3)	0.0857(1)
C10	0.33062(1)	1.00550(1)	-0.2039(3)	0.0764(9)
N11	0.36929(7)	1.03869(7)	-0.1731(2)	0.0601(6)
C12	0.41524(1)	1.01382(1)	-0.1884(3)	0.0717(8)
C13	0.42075(1)	0.99688(1)	-0.3037(3)	0.0730(8)
C14	0.36423(1)	1.05417(1)	-0.0583(3)	0.0734(8)
C15	0.32040(9)	1.08454(9)	-0.0373(2)	0.0591(7)
C16	0.28924(1)	1.06919(1)	0.0502(2)	0.0641(7)
O17	0.25033(7)	1.09768(7)	0.07558(2)	0.0683(6)
C18	0.24048(1)	1.13879(9)	0.0170(2)	0.0611(7)
C19	0.19918(1)	1.16316(1)	0.0486(3)	0.0786(9)
C20	0.18559(1)	1.20329(1)	-0.0099(3)	0.0883(1)
C21	0.21231(1)	1.21877(1)	-0.0977(3)	0.0755(9)
C22	0.25380(1)	1.19494(9)	-0.1270(2)	0.0628(7)
C23	0.26950(9)	1.15463(8)	-0.0684(2)	0.0539(6)
C24	0.31143(9)	1.12571(9)	-0.0946(2)	0.0550(7)
O25	0.29462(8)	1.03436(8)	0.10809(2)	0.0858(7)
N26	0.34024(8)	1.13734(8)	-0.18323(2)	0.0663(6)
C27	0.36309(9)	1.18176(1)	-0.2033(3)	0.0657(8)
C28	0.38203(1)	1.19040(1)	-0.3070(3)	0.0849(1)
C29	0.40611(1)	1.23175(2)	-0.3290(4)	0.1079(1)
C30	0.41187(1)	1.26587(2)	-0.2501(5)	0.1251(2)
C31	0.39303(1)	1.25863(1)	-0.1468(4)	0.1120(1)
C32	0.36887(1)	1.21628(1)	-0.1227(3)	0.0829(9)

Table 1. Crystal data and structure refinement table.

Empirical formula	$C_{27}\ H_{27}\ N_3\ O_2$
Formula weight	425.52
Temperature	293(2) K
Wavelength	0.71073 Å
Crystal system	Tetragonal
Spacegroup	$I41/a$
Cell dimensions	
	$a = 27.7990(13)$Å
	$b = 27.7990(6)$Å
	$c = 12.1800(8)$Å
Volume	9412.5(7)Å^3
Z	16
Density(calculated)	1.201 Mg/m^3
Absorption coefficient	0.077 mm^{-1}
$F_(000)$	3616
Crystal size	$0.3 \times 0.3 \times 0.3$ mm
θ range for data collection	2.07° to 24.98°
Index ranges	$-32 \leq h \leq 32$
	$-23 \leq k \leq 23$
	$-14 \leq l \leq 13$
Reflections collected	7924
Independent reflections	4133 [R(int) = 0.0219]]
Absorption correction	None
Data / restraints / parameters	4133 / 0 / 290
Goodness-of-fit on F^2	1.060
Final R indices [$I > 2\sigma(I)$]	$R1 = 0.0692$, $wR2 = 0.2093$
R indices (all data)	$R1 = 0.1007$, $wR2 = 0.2491$
Extinction coefficient	0.0023(6)
Largest diff. peak and hole	0.780 and -0.210 e.Å^{-3}
Refinement method	Full-matrix least-squares on F^2
Measurement	DIPLabo
Program system	Denzo
Structure determination	SHELXS-97
Refinement	SHELXL-97

5.12 ANALYTICAL DATA

4-ANILINO-3-[(4-METHYL PIPERAZIN-1-YL) METHYL]-2*H*-CHROMEN-2-ONE (DNJ-1001) : IR (KBr, cm^{-1}): 3381 (-NH), 3074 (Ar-H, str), 2962 (-CH_3), 2809 (-CH_2), 1710 (>CO), 1562, 1540, 1470, 1462 (Ar-H, ben), 1336 (C-N, Ar, 3°), 1306 (C-N, Ar, 2°), 1055 (C-O-C); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 2.33 (4H, s), 2.60 (7H, s), 3.77 (2H, s), 6.97 (3H, m, *J* = 1.12 Hz, *J* = 0.80 Hz), 7.11 (1H, s), 7.33 (4H, m, *J* = 0.44 Hz, *J* = 1.28 Hz), 7.43 (1H, m, *J* = 1.52 Hz, *J* = 1.44 Hz), 10.30 (1H, s); ^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm) 162.70, 153.69, 152.09, 142.07, 131.22, 129.37, 126.12, 123.45, 122.64, 121.02, 117.26, 115.22, 102.72, 55.36, 54.36, 52.18, 46.05; MS m/z = 349 (M^+); Anal. Calcd. for $C_{21}H_{23}N_3O_2$: C, 72.18; H, 6.63; N, 12.03. Found: C, 72.16; H, 6.65; N, 12.01.

4-ANILINO-3-[(4-ETHYL PIPERAZIN-1-YL) METHYL]-2*H*-CHROMEN-2-ONE (DNJ-1002): IR (KBr, cm^{-1}): 3379 (-NH), 3075 (Ar-H, str), 2969 (-CH_3), 2810 (-CH_2), 1708 (>CO), 1556, 1539, 1501, 1470 (Ar-H, ben), 1337 (C-N, Ar, 3°), 1306 (C-N, Ar, 2°), 1052 (C-O-C); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.13 (3H, s), 2.48 (4H, m), 2.62 (6H, s), 3.77 (2H, s), 6.93 (3H, m, *J* = 0.903 Hz, *J* = 1.206 Hz), 7.11 (1H, s), 7.32 (4H, m, *J* = 3.807 Hz, *J* = 1.173 Hz, *J* = 0.912 Hz), 7.44 (1H, m, *J* = 2.325 Hz, *J* = 1.059 Hz, *J* = 1.308 Hz), 10.37 (1H, s); ^{13}C NMR (300 MHz, $CDCl_3$): δ (ppm) 163.06, 154.11, 152.48, 142.44, 131.56, 129.74, 126.51, 123.79, 123.00, 121.40, 117.64, 115.67, 103.24, 54.81, 53.44, 52.63, 12.44; MS m/z = 363 (M^+); Anal. Calcd. for $C_{22}H_{25}N_3O_2$: C, 72.70; H, 6.93; N, 11.56. Found: C, 72.71; H, 6.91; N, 11.53.

4-ANILINO-3-[(4-BENZYL PIPERAZIN-1-YL) METHYL]-2*H*-CHROMEN-2-ONE (DNJ-1003): IR (KBr, cm^{-1}): 3385 (-NH), 3079 (Ar-H, str), 2967 (-CH_3), 2811 (-CH_2), 1706 (>CO), 1600, 1539, 1570, 1500, 1460 (Ar-H, ben), 1338 (C-N, Ar, 3°), 1303 (C-N, Ar, 2°), 1057 (C-O-C); MS m/z = 425 (M^+); Anal. Calcd. for $C_{27}H_{27}N_3O_2$: C, 76.21; H, 6.40; N, 9.87. Found: C, 76.24; H, 6.38; N, 9.85.

4-[(2-CHLORO PHENYL) AMINO]-3-[(4-ETHYL PIPERAZIN-1-YL) METHYL]-2*H*-CHROMEN-2-ONE (DNJ-1004): IR (KBr, cm^{-1}): 3389 (-NH), 3070 (Ar-H, str), 2966 (-CH_3), 2810 (-CH_2), 1707 (>CO), 1570, 1490, 1470, 1440 (Ar-H, ben), 1336 (C-N, Ar, 3°), 1305 (C-N, Ar, 2°), 1060 (C-O-C), 871 (C-Cl), 755 (1,2-di sub); MS m/z = 397 (M^+); Anal. Calcd. for $C_{22}H_{24}ClN_3O_2$: C, 66.41; H, 6.08; N, 10.56. Found: C, 66.39; H, 6.05; N, 10.53.

4-[(3-CHLORO PHENYL) AMINO]-3-[(4-ETHYL PIPERAZIN-1-YL) METHYL]-2*H*-CHROMEN-2-ONE (DNJ-1005): IR (KBr, cm^{-1}): 3390 (-NH), 3071 (Ar-H, str), 2967 (-CH_3), 2810 (-CH_2), 1706 (>CO), 1572, 1488, 1475, 1440 (Ar-H, ben), 1338 (C-N, Ar, 3°), 1306 (C-N, Ar, 2°), 1076 (C-O-C), 865 (C-Cl), 770 (1,3-di sub); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.13 (3H, s), 2.50 (4H, m), 2.62 (6H, s), 3.76 (2H, s), 6.78 (1H, dd, *J* = 1.635 Hz, *J* = 1.428 Hz), 6.92 (1H, t), 7.05 (2H, m, *J* = 0.957 Hz), 7.21 (1H, s), 7.34 (2H, m, *J* = 1.233 Hz, *J* = 0.723 Hz), 7.48 (1H, dd, *J* = 1.389 Hz, *J* = 1.149 Hz, *J* = 1.362 Hz), 10.41 (1H, s); ^{13}C NMR (300 MHz, $CDCl_3$): δ (ppm) 162.89, 154.05, 151.98, 143.99, 135.41, 131.84, 130.67, 126.30, 123.46, 123.31, 120.77, 118.97, 117.80, 115.53, 105.13, 54.76, 53.43, 52.70, 52.65, 12.43; MS m/z = 397 (M^+); Anal. Calcd. for $C_{22}H_{24}ClN_3O_2$: C, 66.41; H, 6.08; N, 10.56. Found: C, 66.44; H, 6.09; N, 10.55.

4-[(4-CHLORO PHENYL) AMINO]-3-[(4-ETHYL PIPERAZIN-1-YL) METHYL]-2*H*-CHROMEN-2-ONE (DNJ-1006): IR (KBr, cm^{-1}): 3370 (-NH), 3080 (Ar-H, str), 2967 (-CH_3), 2810 (-CH_2), 1706 (>CO), 1572, 1488, 1475, 1440 (Ar-H, ben), 1337 (C-N, Ar, 3°), 1307 (C-N, Ar, 2°), 1050 (C-O-C), 869 (C-Cl), 812 (1,4-di sub); MS m/z = 397 (M^+); Anal. Calcd. for $C_{22}H_{24}ClN_3O_2$: C, 66.41; H, 6.08; N, 10.56. Found: C, 66.40; H, 6.07; N, 10.58.

3-[(4-ETHYL PIPERAZIN-1-YL) METHYL]-4-[(2-FLUORO PHENYL) AMINO]-2*H*-CHROMEN-2-ONE (DNJ-1007): IR (KBr, cm^{-1}): 3381 (-NH), 3070 (Ar-H, str), 2962 (-CH_3), 2811 (-CH_2), 1707 (>CO), 1570, 1484, 1470, 1438 (Ar-H, ben), 1337 (C-N, Ar, 3°), 1306 (C-N, Ar, 2°), 1054 (C-O-C), 990 (C-F), 750 (1,2-di sub); MS m/z = 381 (M^+); Anal. Calcd. for $C_{22}H_{24}FN_3O_2$: C, 69.27; H, 6.34; N, 11.02. Found: C, 69.23; H, 6.38; N, 11.05.

3-[(4-ETHYL PIPERAZIN-1-YL) METHYL]-4-[(4-FLUORO PHENYL) AMINO]-2*H*-CHROMEN-2-ONE (DNJ-1008): IR (KBr, cm^{-1}): 3388 (-NH), 3080 (Ar-H, str), 2967 (-CH_3), 2811 (-CH_2), 1707 (>CO), 1607, 1584, 1500, 1470 (Ar-H, ben), 1335 (C-N, Ar, 3°), 1304 (C-N, Ar, 2°), 1061 (C-O-C), 992 (C-F), 819 (1,4-di sub); MS m/z = 381 (M^+); Anal. Calcd. for $C_{22}H_{24}FN_3O_2$: C, 69.27; H, 6.34; N, 11.02. Found: C, 69.28; H, 6.36; N, 11.04.

3-[(4-ETHYL PIPERAZIN-1-YL) METHYL]-4-[(2-METHYL PHENYL) AMINO]-2*H*-CHROMEN-2-ONE (DNJ-1009): IR (KBr, cm^{-1}): 3389 (-NH), 3079 (Ar-H, str), 2962 (-CH_3), 2809 (-CH_2), 1706 (>CO), 1607, 1584, 1500, 1470 (Ar-H, ben), 1338 (C-N, Ar, 3°), 1307 (C-N, Ar, 2°), 1069 (C-O-C), 755 (1,2-di sub); MS m/z = 377 (M^+); Anal. Calcd. for $C_{23}H_{27}N_3O_2$: C, 73.18; H, 7.21; N, 11.13. Found: C, 73.15; H, 7.24; N, 11.10.

3-[(4-ETHYL PIPERAZIN-1-YL) METHYL]-4-[(3-METHYL PHENYL) AMINO]-2*H*-CHROMEN-2-ONE (DNJ-1010): IR (KBr, cm^{-1}): 3388 (-NH), 3081 (Ar-H, str), 2962 (-CH_3), 2811 (-CH_2), 1707 (>CO), 1600, 1575, 1481, 1465 (Ar-H, ben), 1338 (C-N, Ar, 3°), 1307 (C-N, Ar, 2°), 1072 (C-O-C), 775 (1,3-di sub); MS m/z = 377 (M^+); Anal. Calcd. for $C_{23}H_{27}N_3O_2$: C, 73.18; H, 7.21; N, 11.13. Found: C, 73.20; H, 7.24; N, 11.10.

3-[(4-ETHYL PIPERAZIN-1-YL) METHYL]-4-[(2-METHOXY PHENYL) AMINO]-2*H*-CHROMEN-2-ONE (DNJ-1011): IR (KBr, cm^{-1}): 3380 (-NH), 3070 (Ar-H, str), 2967 (-CH_3), 2809 (-CH_2), 1705 (>CO), 1600, 1584, 1507, 1470 (Ar-H, ben), 1337 (C-N, Ar, 3°), 1305 (C-N, Ar, 2°), 1051 (C-O-C), 758 (1,2-di sub); MS m/z = 393 (M^+); Anal. Calcd. for $C_{23}H_{27}N_3O_3$: C, 70.21; H, 6.92; N, 10.68. Found: C, 70.19; H, 6.95; N, 10.71.

3-[(4-ETHYL PIPERAZIN-1-YL) METHYL]-4-[(3-METHOXY PHENYL) AMINO]-2*H*-CHROMEN-2-ONE (DNJ-1012): IR (KBr, cm^{-1}): 3388 (-NH), 3080 (Ar-H, str), 2967 (-CH_3), 2811 (-CH_2), 1707 (>CO), 1602, 1580, 1483, 1469 (Ar-H, ben), 1336 (C-N, Ar, 3°), 1307 (C-N, Ar, 2°), 1070 (C-O-C), 780 (1,3-di sub); MS m/z = 393 (M^+); Anal. Calcd. for $C_{23}H_{27}N_3O_3$: C, 70.21; H, 6.92; N, 10.68. Found: C, 70.20; H, 6.95; N, 10.67.

3-[(4-ETHYL PIPERAZIN-1-YL) METHYL]-4-{[3-(TRIFLUOROMETHYL) PHENYL] AMINO}-2*H*-CHROMEN-2-ONE (DNJ-1013): IR (KBr, cm^{-1}): 3388 (-NH), 3080 (Ar-H, str), 2967 ($-CH_3$), 2811 ($-CH_2$), 1707 (>CO), 1607, 1571, 1540, 1481 (Ar-H, ben), 1338 (C-N, Ar, 3°), 1305 (C-N, Ar, 2°), 1071 (C-O-C), 956 (C-F), 882 (1,3-di sub); ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.12 (3H, s), 2.49 (4H, m), 2.62 (6H, s), 3.76 (2H, s), 7.03 (2H, m, *J* = 1.155 Hz), 7.13 (1H, s), 7.42 (3H, m, *J* = 1.218 Hz, *J* = 1.296 Hz, *J* = 2.313 Hz), 7.44 (1H, m, *J* = 1.491 Hz, *J* = 1.218 Hz, *J* = 1.464 Hz), 10.54 (1H, s); ^{13}C NMR (300 MHz, $CDCl_3$): δ (ppm) 162.83, 154.04, 151.78, 143.29, 132.90, 132.47, 132.03, 131.60, 129.53, 125.92, 123.59, 122.31, 119.82, 119.77, 119.72, 119.67, 118.70, 117.12, 117.07, 117.02, 116.97, 115.37, 105.57, 54.78, 53.42, 52.73, 52.63, 12.38; MS m/z = 431 (M^+); Anal. Calcd. for $C_{23}H_{24}F_3N_3O_2$: C, 64.03; H, 5.61; N, 9.74. Found: C, 64.07; H, 5.65; N, 9.70.

3-[(4-ETHYL PIPERAZIN-1-YL) METHYL]-4-(OCTYLAMINO)-2*H*-CHROMEN-2-ONE (DNJ-1014): IR (KBr, cm^{-1}): 3381 (-NH), 3078 (Ar-H, str), 2966 ($-CH_3$), 2810 ($-CH_2$), 1705 (>CO), 1600, 1580, 1480, 1469 (Ar-H, ben), 1337 (C-N, Ar, 3°), 1306 (C-N, Ar, 2°), 1055 (C-O-C); MS m/z = 399 (M^+); Anal. Calcd. for $C_{24}H_{37}N_3O_2$: C, 72.14; H, 9.33; N, 10.52. Found: C, 72.18; H, 9.37; N, 10.49.

3-[(4-ETHYL PIPERAZIN-1-YL) METHYL]-4-[(2-FURYL METHYL) AMINO]-2*H*-CHROMEN-2-ONE (DNJ-1015): IR (KBr, cm^{-1}): 3388 (-NH), 3081 (Ar-H, str), 2967 ($-CH_3$), 2811 ($-CH_2$), 1707 (>CO), 1600, 1580, 1480, 1469 (Ar-H, ben), 1338 (C-N, Ar, 3°), 1309 (C-N, Ar, 2°), 1074 (C-O-C); MS m/z = 367 (M^+); Anal. Calcd. for $C_{21}H_{25}N_3O_3$: C, 68.64; H, 6.86; N, 11.44. Found: C, 68.60; H, 6.90; N, 11.47.

5.13 RESULTS AND DISCUSSION

A known method was adopted for the preparation of aryl amino coumarins. As said earlier, unexplored chemistry part of *N*-substituted fused coumarin benzothiazine, novelty and biological importance of targeted compounds inspired us to prepare some biologically important *N*-substituted fused coumarin benzothiazine derivatives. But under acidic condition β-keto ester of coumarin nucleus tautomarize into enol form and hydrogen present at C_3 carbon atom became more acidic than secondary amine (-NH) substituted at C_4 carbon atom. According to principle of Mannich reaction and its mechanism, Mannich reaction takes place on acidic hydrogen atom using secondary amine and formaldehyde solution or paraformaldehyde and the compounds thus prepared are known as Mannich bases. Therefore by carrying out Mannich reaction on aryl amino coumarins, we got *C*-Mannich bases instead of *N*-Mannich bases which was the **first principal interesting investigation** of this study.

In this chapter, different *C*-Mannich bases have been prepared using different aryl amino coumarins and different secondary amines *viz.* 1-ethyl piperazine, 1-methyl piperazine and 1-benzyl piperazine. *C*-Mannich bases of 1-ethyl piperazine with all aryl amino coumarins gave products very rapidly while *C*-Mannich bases of 1-methyl piperazine and 1-benzyl piperazine took 2.5-3 months to fall out which was the **second interesting observation**. Similarly, other secondary amines like piperidine, morpholine etc. were also used on trial & error basis but the product did not fall out till 6-7 months.

There was the vast difference in the solubility of the starting material i.e. aryl amino coumarins and in the products i.e. *C*-Mannich bases. The aryl amino coumarins were soluble in dimethylformamide while the products got dissolved in common organic solvents like acetone and methanol which was the **third interesting observation.** These *C*-Mannich bases were found highly crystalline at the time of isolation and did not require recrystallization though they were recystallized in acetone or methanol. The colour of all the products varied between white to pale yellow at the time of isolation.

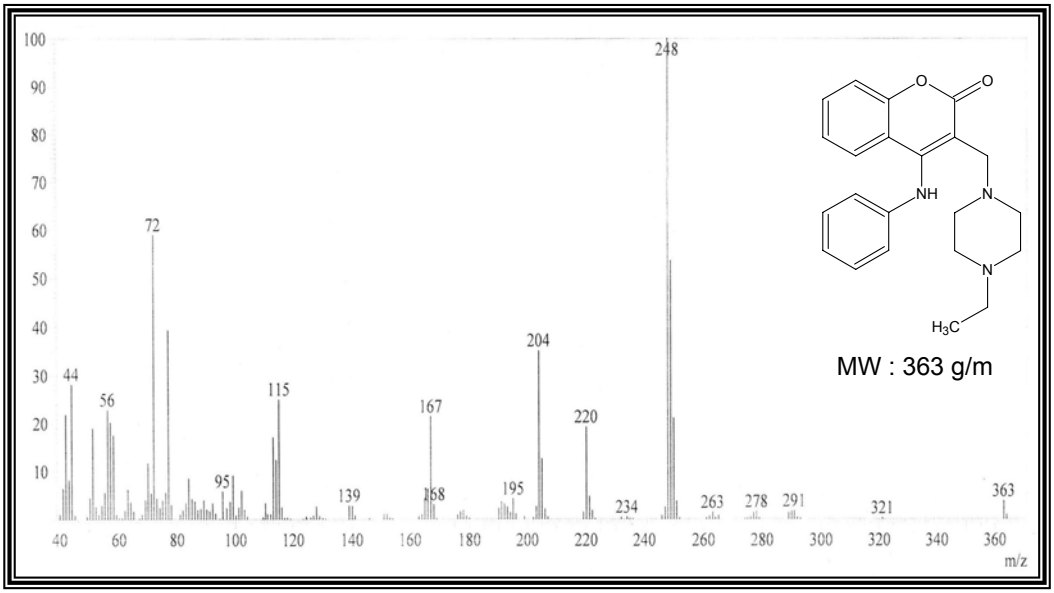
Above outcome of the synthetic aspect was further proved by ^{1}H, ^{13}C NMR (APT 25) and X-ray crystallography. The crystal structure and all data are included.

5.14 CONCLUSION

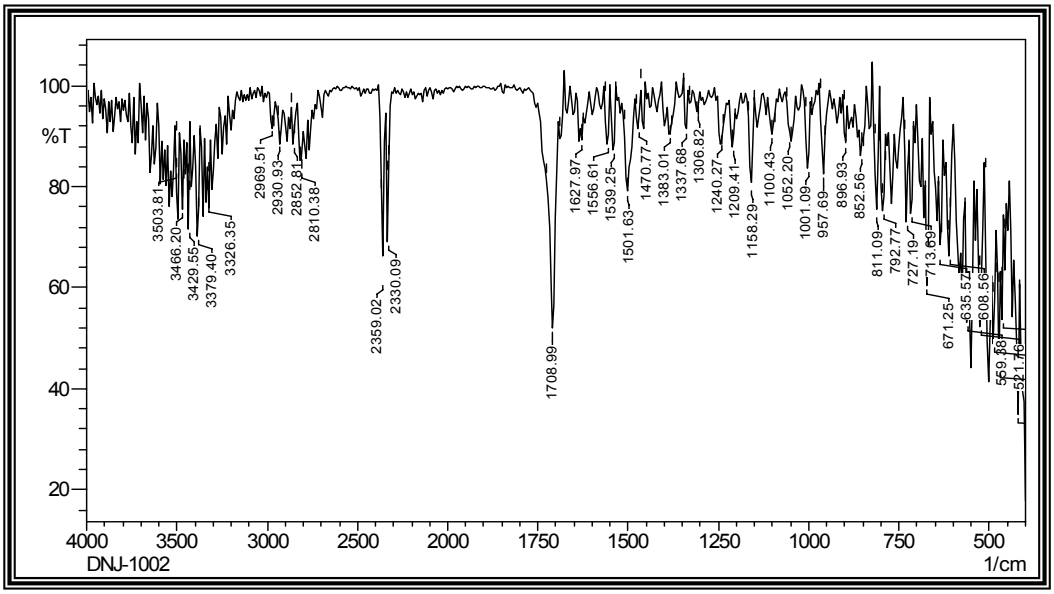
In this chapter, a simple and fast method was developed for the preparation of novel *C*-Mannich bases of aryl amino coumarin using formaldehyde solution and different secondary amines for the first time. APT-25 experiment has proved the possible mechanistic path governing this reaction, which is probably hitherto unknown in literature furthermore no report of other method for optimization for the synthesis of end products is found in literature.

5.15 SPECTRAL REPRESENTATION OF SYNTHESIZED COMPOUNDS

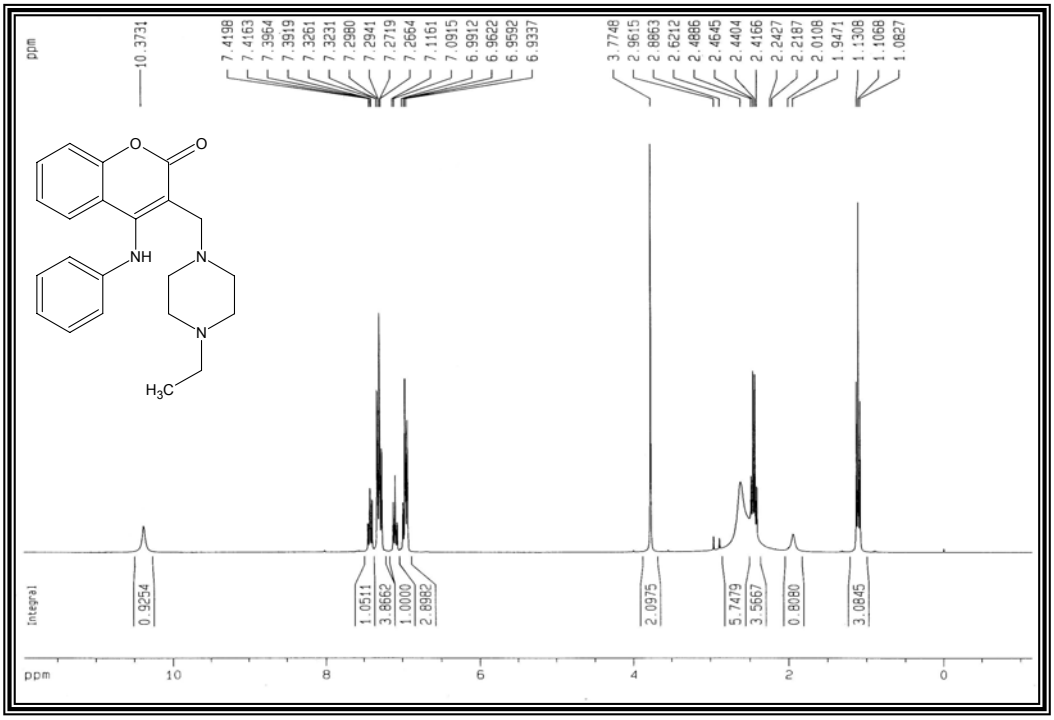
5.15.1 Mass Spectrum of DNJ-1002

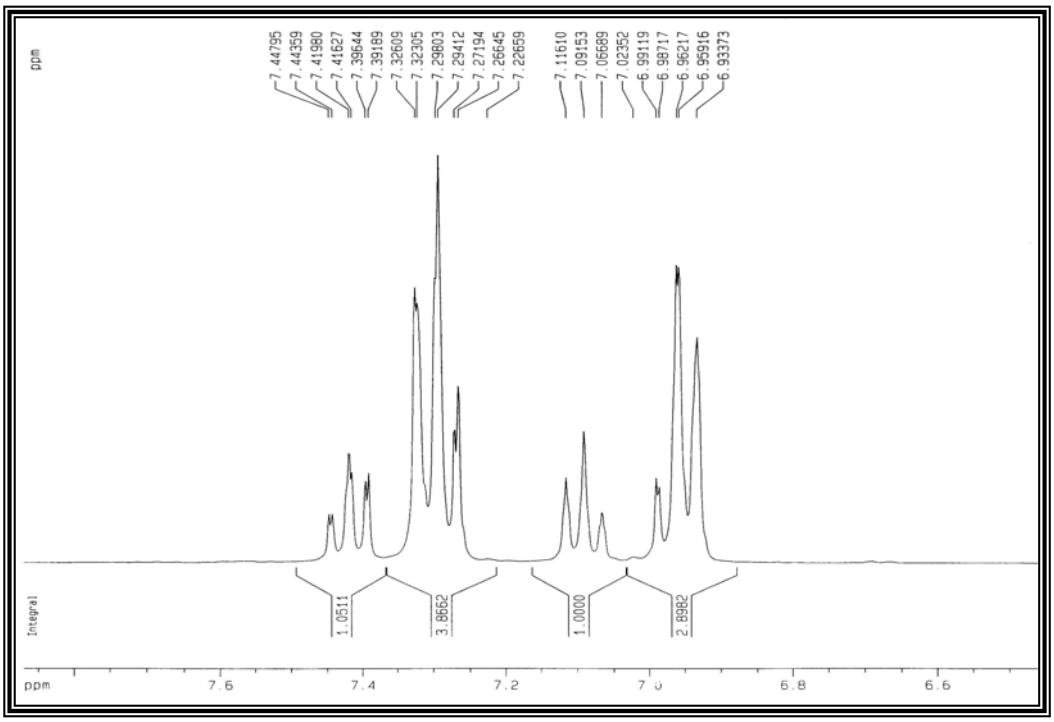


5.15.2 IR Spectrum of DNJ-1002

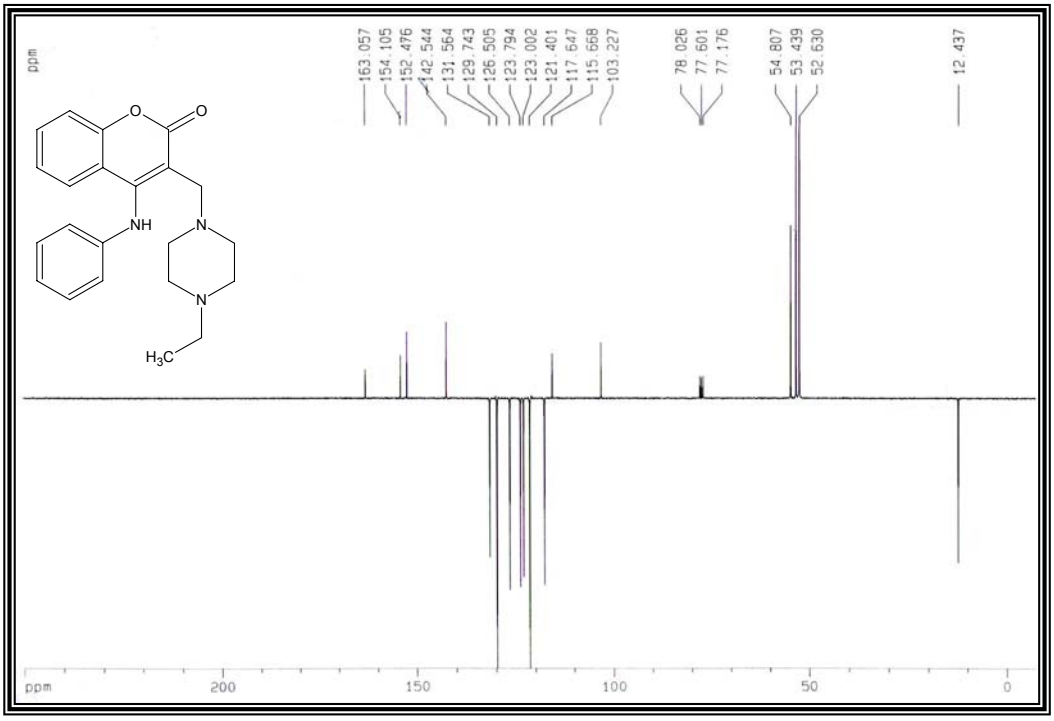


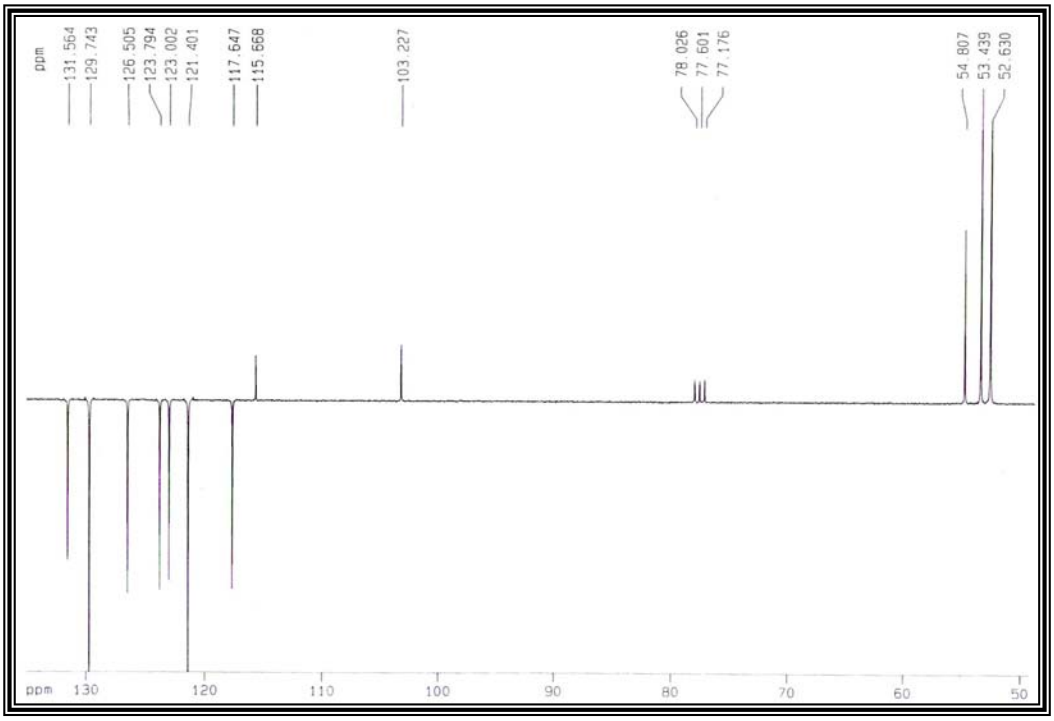
5.15.3 ^{1}H NMR Spectrums of DNJ-1002



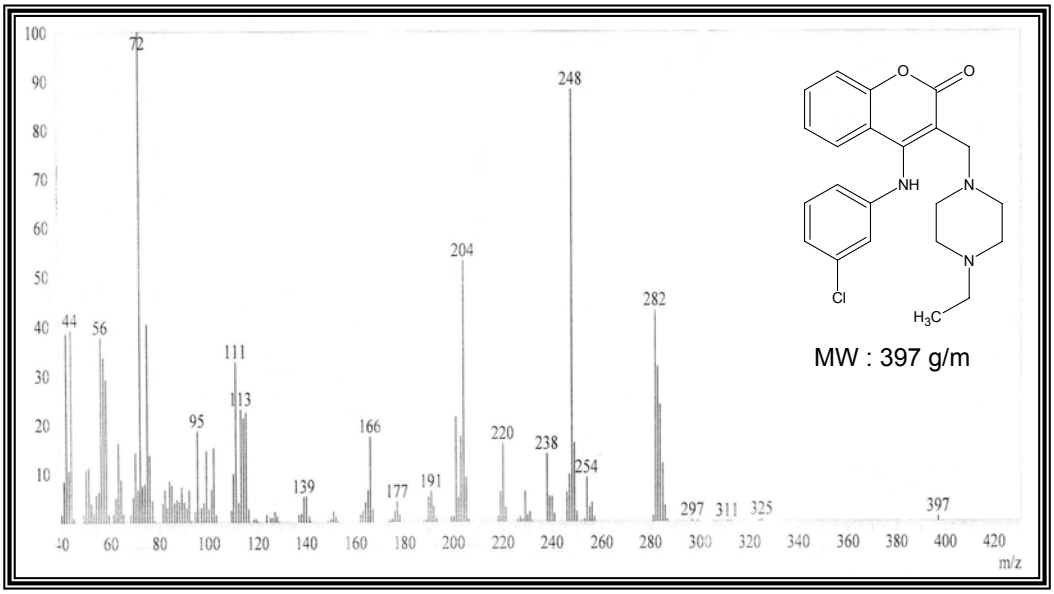


5.15.5 ^{13}C NMR (APT) Spectrums of DNJ-1002

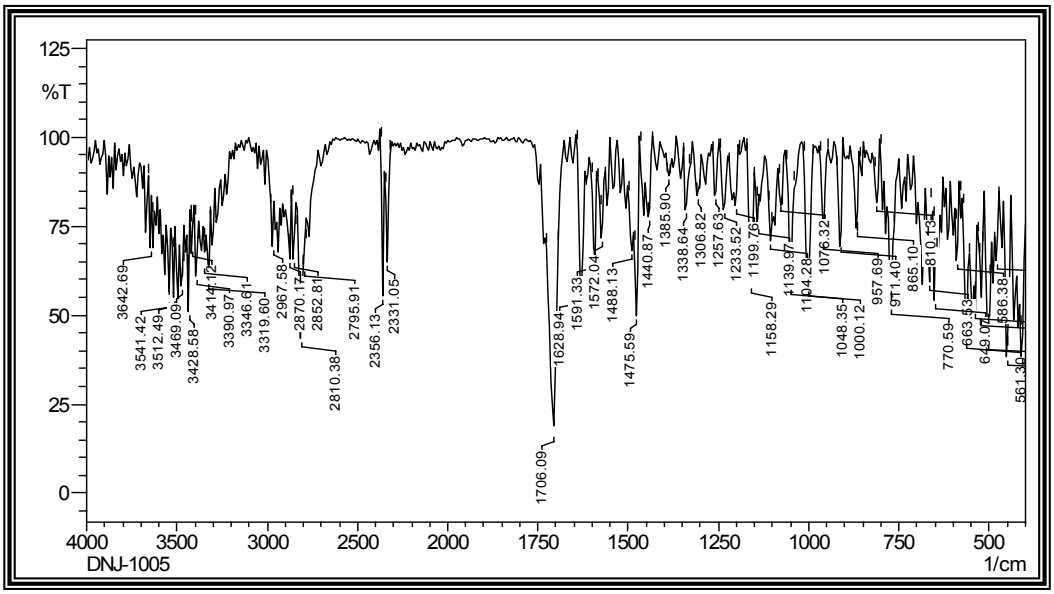




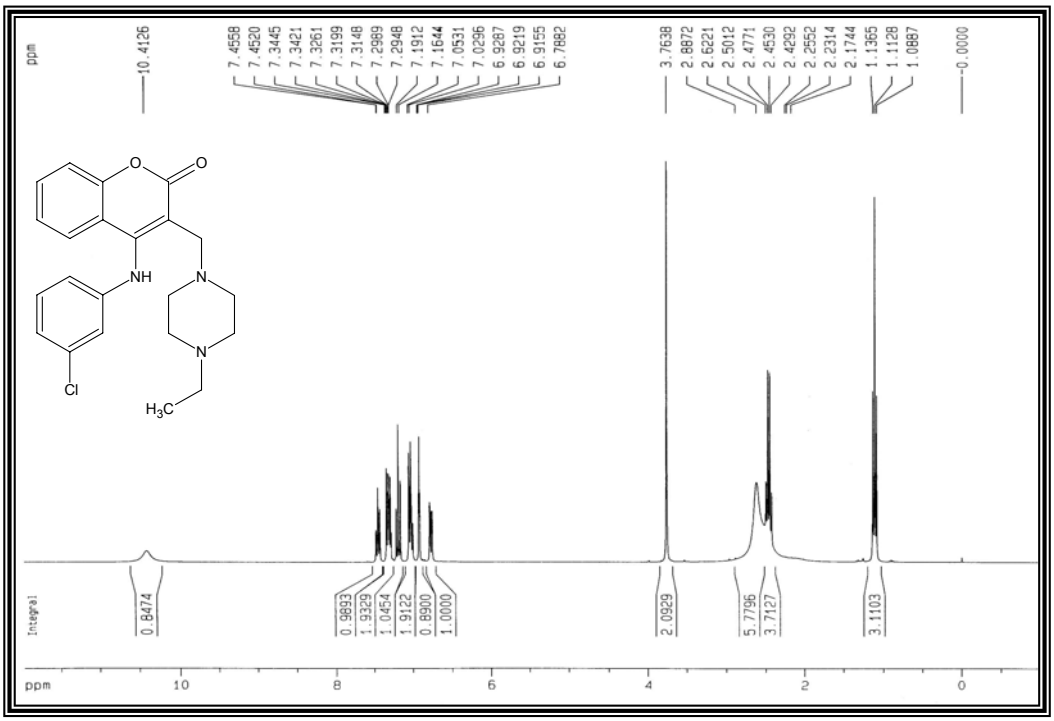
5.15.6 Mass Spectrum of DNJ-1005

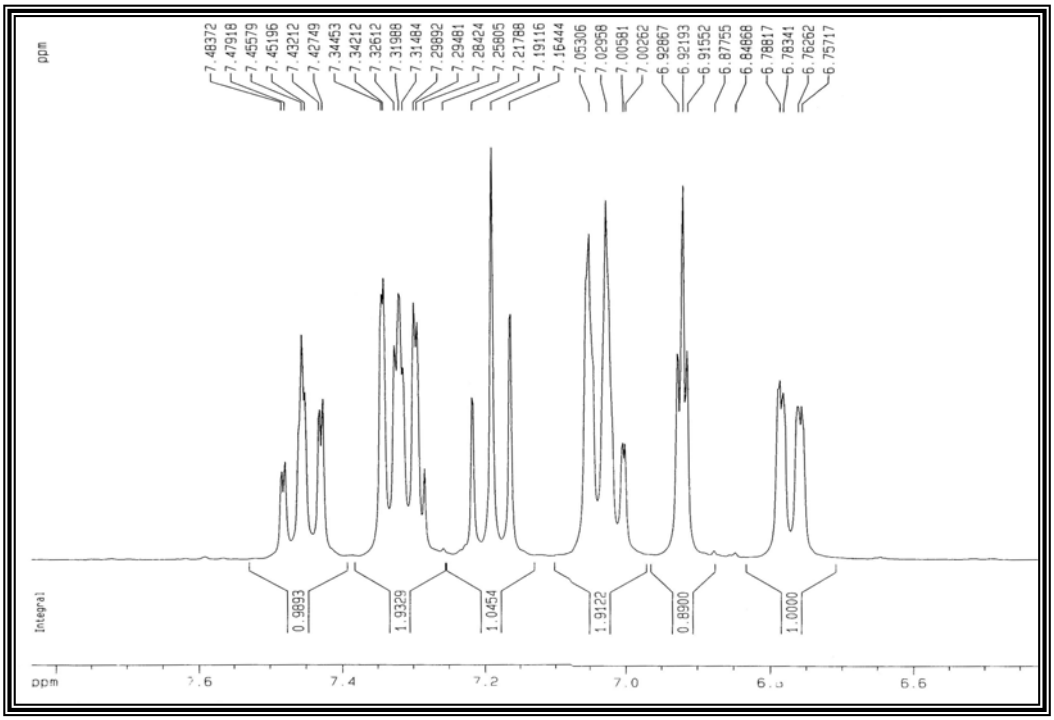


5.15.7 IR Spectrum of DNJ-1005

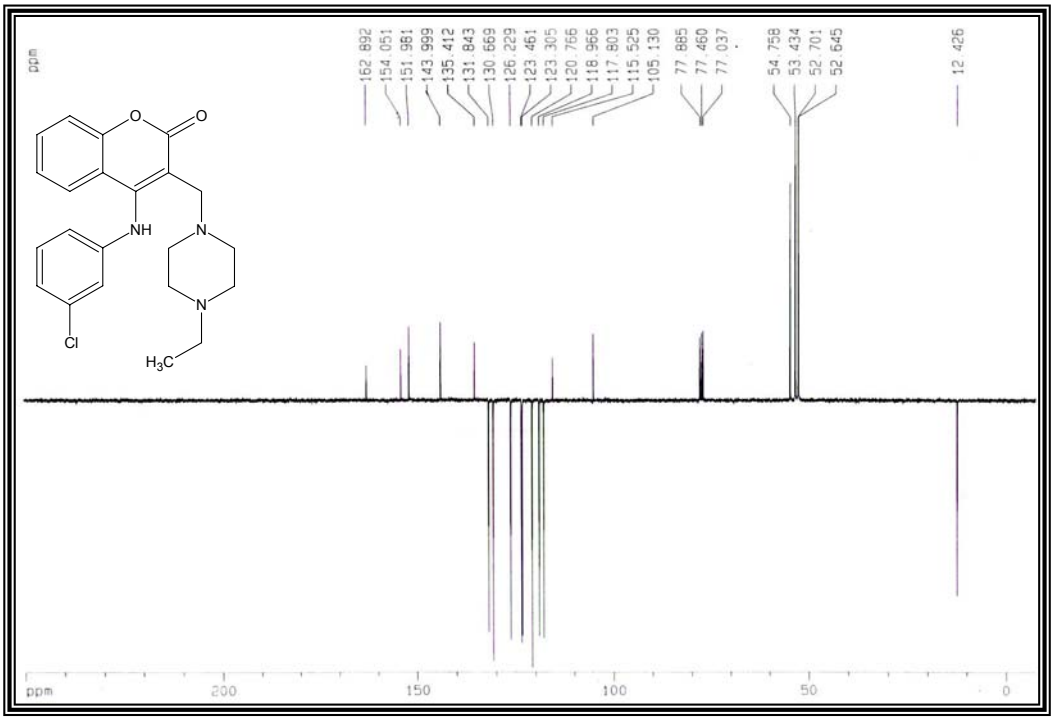


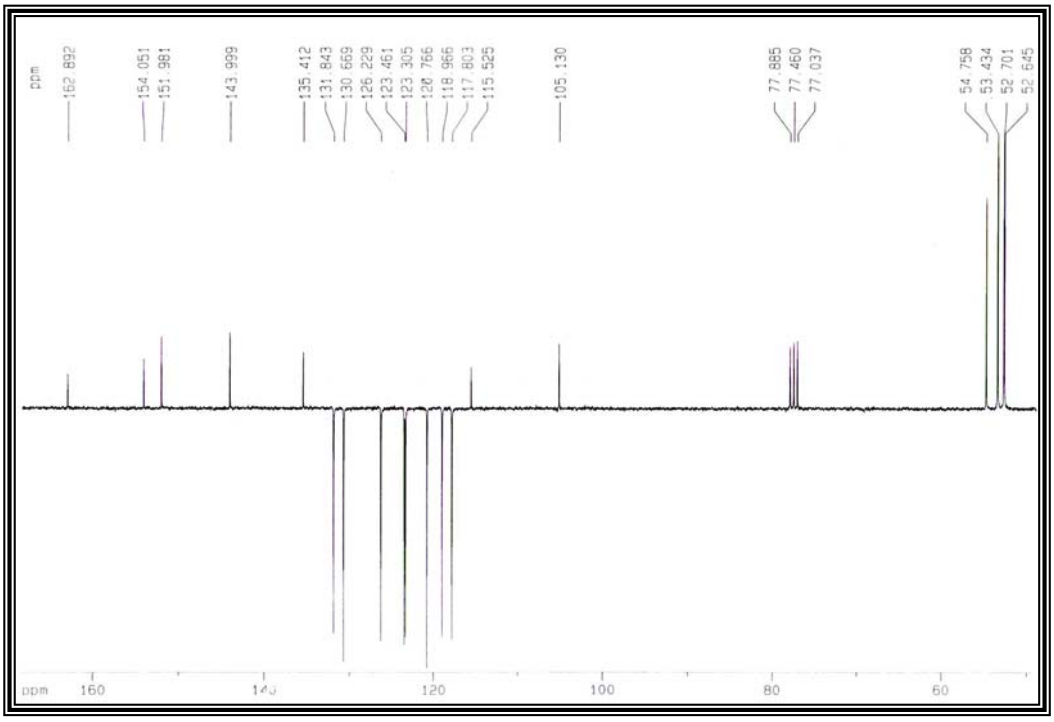
5.15.8 ^{1}H NMR Spectrums of DNJ-1005



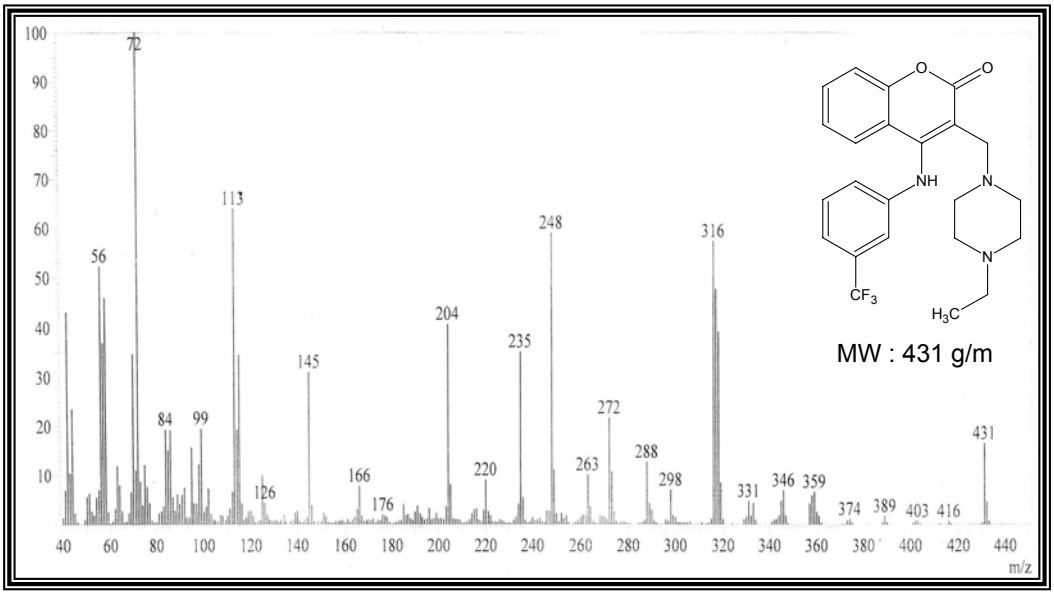


5.15.9 ^{13}C NMR (APT) Spectrums of DNJ-1005

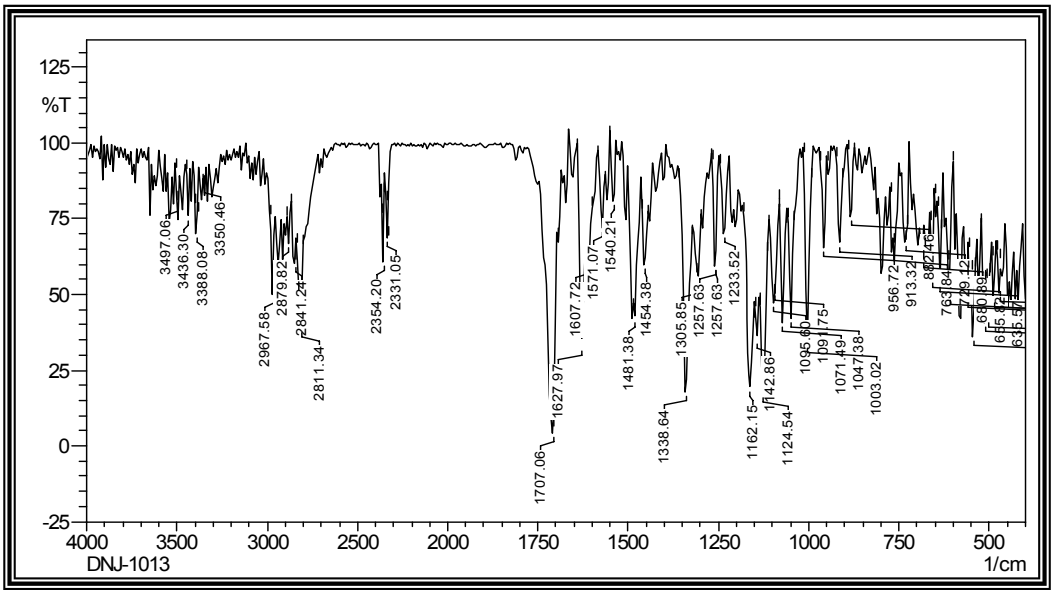




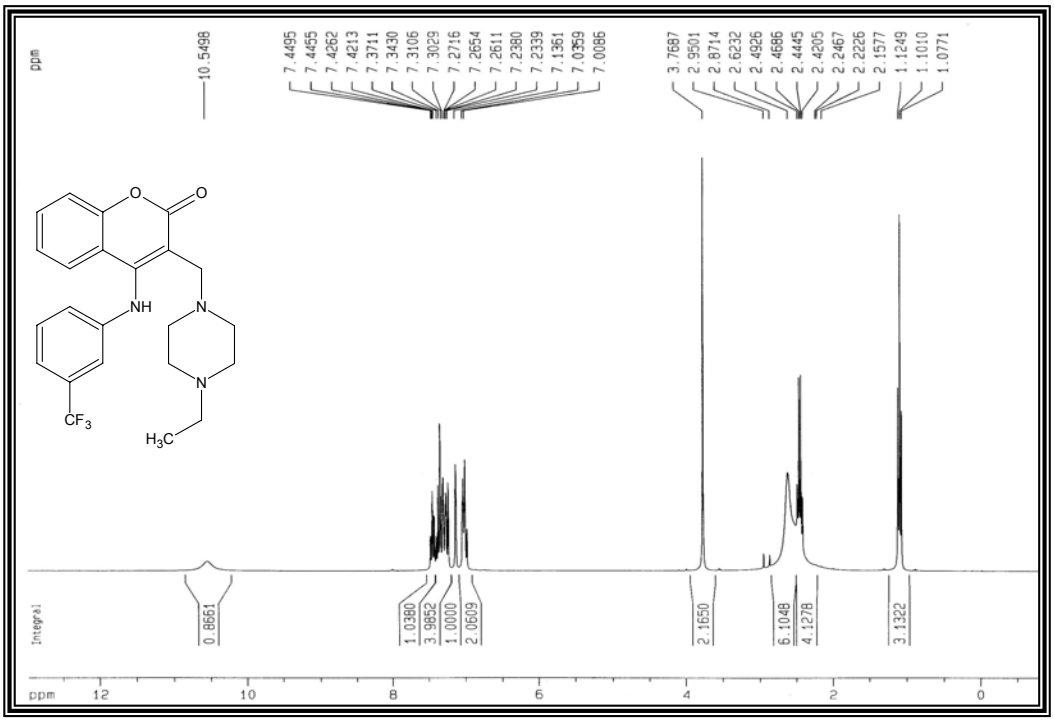
5.15.10 Mass Spectrum of DNJ-1013

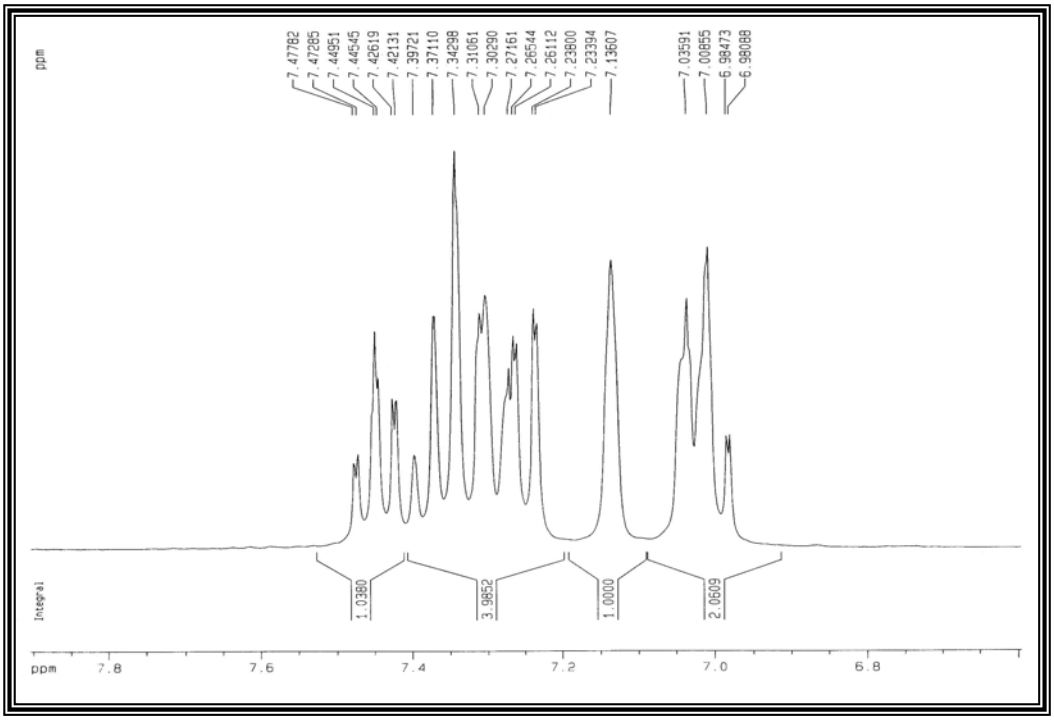


5.15.11 IR Spectrum of DNJ-1013

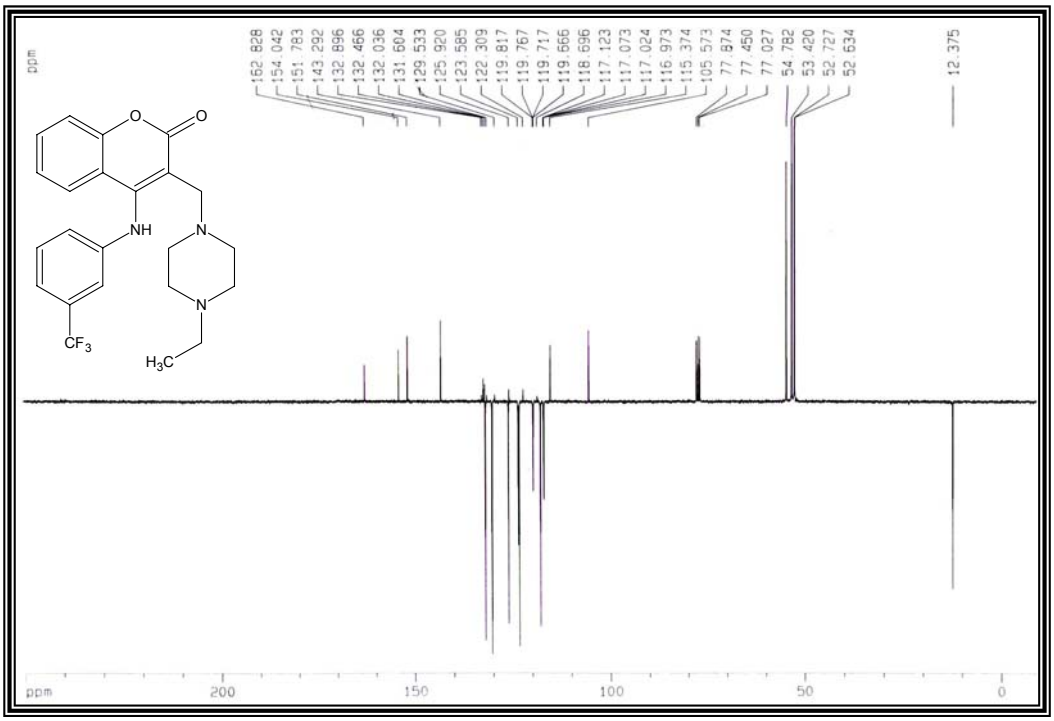


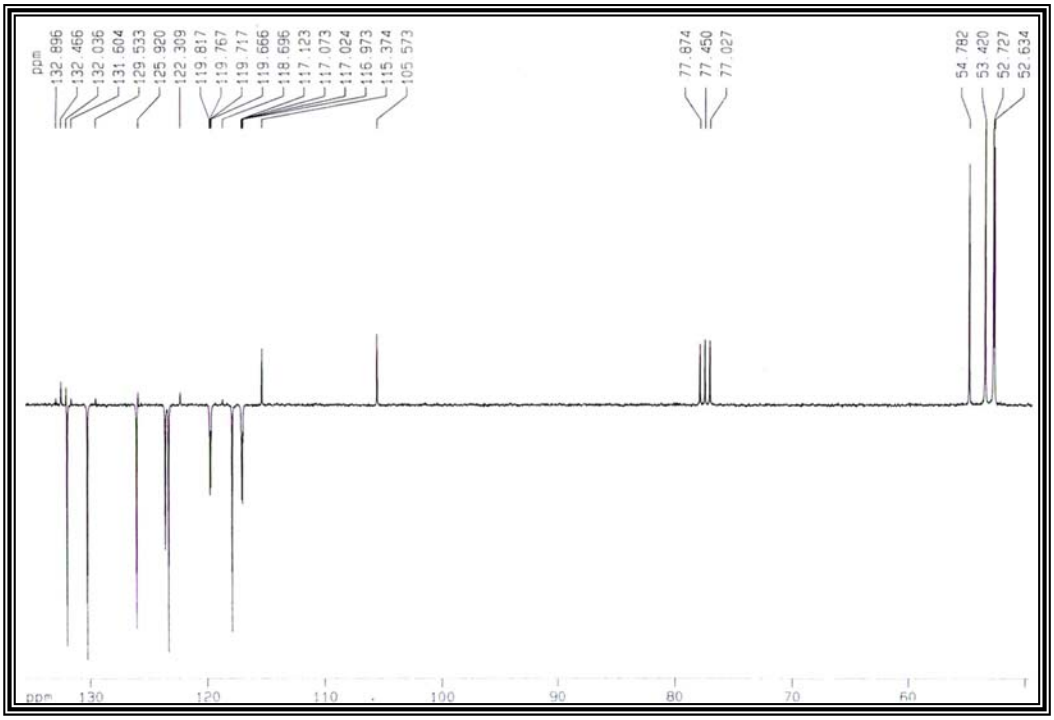
5.15.12 ^{1}H NMR Spectrums of DNJ-1013





5.15.13 ^{13}C NMR (APT 25) Spectrums of DNJ-1013





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CHAPTER – 6

BIOLOGICAL EVALUATION OF SELECTED NEWLY SYNTHESIZED COMPOUNDS

The representative compounds synthesized in earlier chapters were evaluated for biological activity. The biological screening protocols and in silico study of some of the compounds are reported in this chapter.

6.1 ANTIMICROBIAL SCREENING

Antibiotic resistance is a serious concern worldwide as it would result in strains against which currently available antibacterial agents will be ineffective. In general, bacterial pathogens may be classified as either gram-positive or gram-negative pathogens. Antibiotics compounds with effective activity against both gram-positive and gram-negative pathogens are generally regarded as having a broad spectrum of activity. The synthesized compounds were preliminary screened against gram-positive and gram-negative pathogens.

Gram-positive pathogens, for example *Staphylococci*, *Enterococci*, *Streptococci* and *Micobacteria* bacteria are particular because of the development of resistant strain which is difficult to eradicate from the hospital environment once established. Example of such strains are methicillin resistance *Staphylococcus* (MRSA), methicillin resistance coagulase negative *Staphylococci* (MRCNS), penicilline resistance *Streptococcus pnumaniae* and multiplied resistance *Enterococcus faecium*, community acquired pathogens (CAP) and so on.

6.1.1 MINIMUM INHIBITORY CONCENTRATION (*MIC*)

In microbiology, *MIC* is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. An *MIC* is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism.

MICs can be determined by agar or broth dilution methods usually following the guidelines of a reference body such as the CLSI, BSAC or EUCAST. There are several commercial methods available, including the well established Etest strips and the recently launched Oxoid *MIC* Evaluator method.

The Etest system comprises a predefined and continuous concentration gradient of different antimicrobial agents, which when applied to inoculated agar plates and incubated, create ellipses of microbial inhibition. The *MIC* is determined where the ellipse of inhibition intersects the strip, and is easily read off the *MIC* reading scale on the strip.

Clinically, the minimum inhibitory concentrations are used not only to determine the amount of antibiotic that the patient will receive but also the type of antibiotic used, which in turn lowers the opportunity for microbial resistance to specific antimicrobial agents.

The antimicrobial activity of newly synthesized 2-methyl indoline derivatives and isatin derivatives synthesized in Chapter – 1 and Chapter – 2 (i.e. DNJ-101 to DNJ-111, DNJ-210 to DNJ-210, DNJ-301 to DNJ-305, DNJ-401 to DNJ-405, DNJ-501 to DNJ-505 and DNJ-601 to DNJ-605) is reported here in, while the compounds are under investigation for another biological activities.

6.1.2 PROTOCOL FOR ANTIBACTERIAL ACTIVITY

The minimum inhibitory concentrations (*MIC*) of the compounds for the microorganisms were determined by preparing working solution for each compound of concentration of 128 µg/ml after dissolving it in DMSO. Two fold serial dilution of above solution was prepared in duplicate using Muller Hinton Borth, in well tissue culture plate with cover flat bottom wells to give a final volume of 150 and concentration of compound ranging from 64 µg/ml – 0.12 µg/ml. 30 µg/ml of standard suspension of each organism which was prepared with turbidity equivalent to the 1:10 diluted 0.5 McFarland standard with density 10^7 CFU/ml. These 96-well tissue culture plate containing the test

samples and positive and negative controls, were incubated at 37C for 16-18 hours. The wells were visually inspected for growth and were also reat at 630 nm by Automated Micropalte Reader [(EL800) Trinity Biotech] and the *MIC*s were recorded as the lowest concentration of drug which inhibits the growth of bacteria.

MIC (Minium Inhibitory Concentration in vitro activity µg/ml) values of DNJ-101 to DNJ-111 and DNJ-201 to DNJ-210

Sr. No.	Representative Compound	Bp	Se	Sp	Sa1	Ef1
1	DNJ-101	0.5	1	4	8	2
2	DNJ-102	2	0.5	2	16	4
3	DNJ-103	4	4	1	32	2
4	DNJ-104	2	0.5	>16	>16	8
5	DNJ-105	2	2	8	4	>16
6	DNJ-106	4	1	2	ND	32
7	DNJ-107	2	4	1	>8	16
8	DNJ-108	2	0.5	4	>32	8
9	DNJ-109	1	2	16	16	4
10	DNJ-110	2	8	32	8	ND
11	DNJ-111	4	8	>32	ND	8
12	Linezolid	1	2	0.5	4	4

Sr. No.	Representative Compound	Bp	Se	Sp	Sa1	Ef1
1	DNJ-201	2	4	1	8	16
2	DNJ-202	1	8	4	8	8
3	DNJ-203	0.5	>16	2	16	8
4	DNJ-204	4	8	8	ND	8
5	DNJ-205	8	8	4	>32	>32
6	DNJ-206	2	2	2	64	64
7	DNJ-207	2	ND	2	ND	4
8	DNJ-208	4	4	2	8	16
9	DNJ-209	>8	2	4	4	>64
10	DNJ-210	16	>16	8	8	32
11	Linezolid	1	2	0.5	4	4

ND = Not Done

Abbreviations

Bp : *Bacillus pumilus* MTCC 1607

Se : *Staphylococcus epidermidis* MTCC 155

Sp : *Staphylococcus pyogenes* MTCC 442

Sa1 : *Staphylococcus aureus* MTCC 96

Ef1 : *Enterococcus faecalis* MTCC 439

MIC (Minium Inhibitory Concentration in vitro activity µg/ml) values of DNJ-301 to DNJ-305, DNJ-401 to DNJ-405, DNJ-501 to DNJ-505 and DNJ-601 to DNJ-605

Sr. No.	Representative Compound	Bp	Se	Sp	Sa1
1	DNJ-301	8	16	>8	64
2	DNJ-302	16	8	16	32
3	DNJ-303	16	32	>64	>32
4	DNJ-304	ND	16	32	16
5	DNJ-305	8	4	2	>8
6	Linezolid	4	4	4	2

Sr. No.	Representative Compound	Bp	Se	Sp	Sa1
1	DNJ-401	ND	ND	ND	4
2	DNJ-402	>32	>32	4	32
3	DNJ-403	4	8	16	>8
4	DNJ-404	2	16	>16	4
5	DNJ-405	0.5	ND	ND	ND
6	Linezolid	4	4	4	2

Sr. No.	Representative Compound	Bp	Se	Sp	Sa1
1	DNJ-501	4	16	64	4
2	DNJ-502	8	32	16	8
3	DNJ-503	16	64	>8	16
4	DNJ-504	ND	16	ND	ND
5	DNJ-505	16	4	>16	8
6	Linezolid	4	4	4	2

Sr. No.	Representative Compound	Bp	Se	Sp	Sa1
1	DNJ-601	8	ND	32	8
2	DNJ-602	ND	8	8	4
3	DNJ-603	64	16	16	16
4	DNJ-604	32	ND	ND	>32
5	DNJ-605	16	32	8	8
6	Linezolid	4	4	4	2

ND = Not Done

6.1.3 RESULTS AND DISCUSSION

From the above mentioned *MIC* values of antibacterial activity of DNJ-101 to DNJ-111, it could be concluded that DNJ-101, DNJ-102, DNJ-104 and DNJ-108 were found to be highest potent with the *MIC* value of 0.5 µg/ml against the *Bacillus pumilus* MTCC 1607 and *Staphylococcus epidermidis* MTCC 155 respectively, which were having more inhibitory effect than the standard drug Linezolid. DNJ-102 to DNJ-111 were also found to have good inhibitory effect with the *MIC* value between 1-8 µg/ml on both of the bacterial strains. Thus it could be concluded that compounds having subunits like piperidine, morpholine, piperazine etc and alkyl chains having one to four carbons were found to possess good antibacterial activity.

While DNJ-104, DNJ-109, DNJ-110 and DNJ-111 were found to be moderate active with the higher *MIC* values (i.e. >16, 16, 32 and >32 µg/ml respectively) against *Staphylococcus pyogenes* MTCC 442. DNJ-102 to DNJ-104 and DNJ-107 to DNJ-109 were found to be moderate active with the higher *MIC* values (i.e. 16, 32, >16 µg/ml and >8, >32, 16 µg/ml) against *Staphylococcus aureus* MTCC 96. DNJ-105 to DNJ-107 were found to be moderate active with the higher *MIC* values (i.e. >16, 32 and 16 µg/ml) against *Enterococcus faecalis* MTCC 439. Thus rests of the compounds were found to be good active with the *MIC* value between 1-8 µg/ml against all the three bacterial strains. From above data it is clear that all the compounds are good active against *Bacillus pumilus* MTCC 1607 and *Staphylococcus epidermidis* MTCC 155 while they are moderate active against

Staphylococcus pyogenes MTCC 442, *Staphylococcus aureus* MTCC 96 and *Enterococcus faecalis* MTCC 439.

In case of second series of compounds (i.e. DNJ-201 to DNJ-210), only one compound DNJ-203 was found to possess highest potency and lowest *MIC* value (i.e. 0.5 µg/ml) against *Bacillus pumilus* MTCC 1607 which was having more inhibitory effect than the standard drug Linezolid. Thus the compound possessing trifluoromethyl group has more inhibitory effect than the other functional groups and shows highest antibacterial activity. While the compound having chloro group possesses second lowest *MIC* value (i.e. 1 µg/ml) against *Bacillus pumilus* MTCC 1607. In short, molecules having halogen group show more inhibition to the growth of afore mentioned bacterias. Rests of the compounds were found to be good active against the same bacterial strain. DNJ-209 and DNJ-210 were found to be moderate active against *Bacillus pumilus* MTCC 1607 with the *MIC* values >8 and 16 µg/ml respectively. All the compounds of this series were found to be good active against *Staphylococcus pyogenes* MTCC 442 with the *MIC* value between 1-8 µg/ml. All these compounds did not show good inhibitory effect against rests of the three bacterial strains i.e. *Staphylococcus epidermidis* MTCC 155, *Staphylococcus aureus* MTCC 96 and *Enterococcus faecalis* MTCC 439, only few compounds were found to possess good activity.

Results of antibacterial screening of DNJ-301 to DNJ-305 showed that all the compounds were moderate active against all the four bacterial strains i.e. *Bacillus pumilus* MTCC 1607, *Staphylococcus epidermidis* MTCC 155, *Staphylococcus pyogenes* MTCC 442 and *Staphylococcus aureus* MTCC 96. Only one compound, DNJ-305 was found to possess good inhibitory effect with the *MIC* values of 4 µg/ml and 2 µg/ml on *Staphylococcus epidermidis* MTCC 155 and *Staphylococcus pyogenes* MTCC 442 respectively. Thus long alkyl chain possessing three carbon atoms on nitrogen atom of indoline nucleus having secondary amine on the another end did not affect the inhibition of the bacterial growth, only the compound (DNJ-305) having benzyl piperazine subunit could show good inhibition.

In case of DNJ-401 to DNJ-405, DNJ-405 was found to possess highest potency and lowest *MIC* value of 0.5 µg/ml against *Bacillus pumilus* MTCC 1607 while DNJ-404 was found to possess second lowest *MIC* value of 2 µg/ml and DNJ-403 was found to possess the *MIC* value of 4 µg/ml against the same bacterial strain. All these compounds were found to be moderate active against *Staphylococcus epidermidis* MTCC 155. DNJ-402 had good inhibitory effect on *Staphylococcus pyogenes* MTCC 442 with the *MIC* value of 4 µg/ml, where DNJ-401 and DNJ-404 were found to possess good antibacterial activity with *MIC* value of 4 µg/ml against *Staphylococcus aureus* MTCC 96. Rests of the compounds were found to be moderate active against all the bacterial strains. Thus the compounds possessing $–CH_2$-CO group on nitrogen atom in indoline nucleus were found to be comparatively more active than the compounds possessing three carbon-alkyl chains on nitrogen atom in indoline nucleus. While the scenario was opposite in case of isatin moiety, where the compounds possessing three carbon-alkyl chains on nitrogen atom in isatin nucleus were found to be comparatively more active than the compounds possessing $–CH_2$-CO group on nitrogen atom in isatin nucleus.

In case of DNJ-501 to DNJ-505 only two compounds i.e. DNJ-501 and DNJ-505 were found to be good active against *Bacillus pumilus* MTCC 1607, *Staphylococcus aureus* MTCC 96 and *Staphylococcus epidermidis* MTCC 155 respectively with the *MIC* value of 4 µg/ml. Rests of the compounds were found to be moderate active against all the four bacterial strains.

Results of antibacterial screening of DNJ-601 to DNJ-605 showed that only one compound DNJ-602 found to possess good antibacterial activity against *Staphylococcus aureus* MTCC 96 with the *MIC* value of 4 µg/ml. While rests of the compounds found to be moderate active against all the four bacterial strains.

6.2 CYTOTOXICITY ASSAY *

Importance of 2-indolinone derivatives as anticancer and especially as tyrosine kinase inhibitors has been discussed in introduction part of Chapter – 3 under the title "Synthetic oxindoles as enzyme inhibitiors". Thus indolinone derivatives (DNJ-1301 to DNJ-1305, DNJ-1401 to DNJ-1405 and DNJ-1501 to DNJ-1505) were screened *in vitro* for their cytotoxic activity at 1-100 µM concentration on a human stomach cancer cell line (Hu Tu 80), breast cancer cell line (MDA.MB.453) and lung cancer cell line (L132). Briefly, a three-day MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] *in vitro* cytotoxicity assay was performed. This test is based on the principle of uptake of MTT, a tetrazolium salt, by the metabolically active cells where it is metabolized by active mitochondria into a blue colored formazan product that is read spectrophotometrically. MTT was dissolved in phosphate buffered saline at a pH of 7.4 to obtain an MTT concentration of 5 mg/ml; the resulting mixture was filtered through a 0.22-mm filter to sterilize and remove a small amount of insoluble residue. A total of 10,000 cells were seeded in a 96-well culture plate and incubated with various concentrations of indolinone derivatives in a CO_2 incubator for 72 hours. Control cells, not treated with indolinone derivatives were similarly incubated. The assay was terminated after 72 hours by adding 125 mg (25 ml) MTT to each well, followed by incubation for 3 hours, and finally added 50 ml of 10% SDS-0.01 N HCl to each well to lyse the cells and dissolve formazan. After incubating for 1 hour, the plate was read spectrophotometrically at 540 nm and the cytotoxicity percentage calculated using the formula: cytotoxicity percentage = $(1-X/R_1) \times 100$, where X = (absorbance of treated sample at 540 nm) - (absorbance of blank at 540 nm) and R_1 = absorbance of control sample at 540 nm.

Results of the cytotoxic assay for above mentioned compounds are summarized in the table given on the next page.

* The results are obtained from Dabur Research Foundation, Ghaziabad.

ED_{50} (µg/ml) of DNJ series on Stomach, Breast, Lung cancer cell lines.

Sr. No.	Representative Compound	Cell Lines		
		Hu Tu 80 (Stomach)	MDA.MB.453 (Breast)	L132 (Lung)
1	DNJ-1301	42	83	>100
2	DNJ-1302	>100	>100	>100
3	DNJ-1303	>100	78	93
4	DNJ-1304	32	33	30
5	DNJ-1305	37	52	>100
6	DNJ-1401	38	35	>100
7	DNJ-1402	49	>100	>100
8	DNJ-1403	>100	>100	>100
9	DNJ-1404	73	61	>100
10	DNJ-1405	>100	>100	74
11	DNJ-1501	>100	>100	>100
12	DNJ-1502	39	>100	>100
13	DNJ-1503	>100	81	>100
14	DNJ-1504	89	61	>100
15	DNJ-1505	30	50	84

6.2.1 RESULTS AND DISCUSSION

From the above mentioned ED_{50} values, it could be concluded that all the compounds possess cytotoxic activity. All the compounds were tested *in vitro* against three cancer cell lines (i.e. stomach cancer cell line-Hu Tu 80, breast cancer cell line-MDA.MB.453 and lung cancer cell line-L132).

In case of DNJ-1301 to DNJ-1305, DNJ-1304 was found to possess highest potency and lowest ED_{50} value against all the three cancer cell lines (i.e. 32, 33 and 30 µg/ml against stomach cancer cell line-Hu Tu 80, Breast cancer cell line-MDA.MB.453 and Lung cancer cell line-L132 respectively). DNJ-1305 also showed good cytotoxicity activity (ED_{50} : 37 µg/ml) against stomach cancer cell line-Hu Tu 80, while it showed moderate (ED_{50} : 52 µg/ml) cytotoxic activity against breast cancer cell line-MDA.MB.453 and it was found less active (ED_{50} : >100 µg/ml) against lung cancer cell line-L132. DNJ-1301 was found to be moderate active (ED_{50} : 42 µg/ml) against stomach cancer cell line while it was found to be moderate active (ED_{50} : 83 µg/ml) against breast cancer cell line and less active (ED_{50} : >100 µg/ml) against

lung cancer cell line. DNJ-1302 was found to be less active against all the three cancer cell lines while DNJ-1303 was found to be moderate active against breast cancer cell line and less active against rests of the two cancer cell lines. Thus from above results it could be summarized that the compound having *N*-ethyl piperazine subunit (i.e. DNJ-1304) found to be most potent molecule against all the three cancer cell lines while the compound having piperidine subunit (i.e. DNJ-1302) did not show good cytotoxic activity. Furthermore the compound having *N*-benzyl piperazine subunit (i.e. DNJ-1305) found to be good active against stomach cancer cell line while it found to be moderate active against breast cancer cell line but its activity against lung cancer cell line was less. The compound having morpholine subunit (i.e. DNJ-1301) found to be good active against stomach cancer cell line while it found to be moderate active against breast cancer cell line but its activity against lung cancer cell line was less. Moreover, the compound having *N*-methyl piperazine subunit (i.e. DNJ-1303) found to be less active against stomach and lung cancer cell line while it found to be moderate active against breast cancer cell line.

In case of DNJ-1401 to DNJ-1405, DNJ-1401 was found to be good active against stomach cancer cell line and breast cancer cell line with the ED_{50} values 38 and 35 µg/ml respectively but it was found less active against lung cancer cell line with ED_{50} value more than 100 µg/ml. DNJ-1402 was found to be moderate active (ED_{50} : 49 µg/ml) against stomach cancer cell line while it was found to be less active (ED_{50} : >100 µg/ml) against rests of the two cancer cell lines. DNJ-1403 was found to be less active against all the three cancer cell lines. DNJ-1404 was found to be moderate active (ED_{50} : 73 µg/ml) against stomach (ED_{50} : 61 µg/ml) and breast cancer cell line while it was found to be less active (ED_{50} : >100 µg/ml) against lung cancer cell line. DNJ-1405 was found to be less active (ED_{50} : >100 µg/ml) against stomach and breast cancer cell lines while it was found to be moderate active (ED_{50} : 74 µg/ml) against lung cancer cell line. Thus from above results it could be summarized that the compound having morpholine subunit (i.e. DNJ-1401) possess good cytotoxic activity against stomach and breast cancer cell lines while it showed less activity against lung cancer cell line. Furthermore the

compound having piperidine subunit (i.e. DNJ-1402) was found to be moderate active against stomach cancer cell line while it was found to be less active against rests of the two cancer cell lines. Moreover the compound having *N*-benzyl piperazine subunit (i.e. DNJ-1405) was found to be moderate active against lung cancer cell line while it was found to be less active against rests of the two cancer cell lines. The compound having *N*-methyl piperazine subunit (i.e. DNJ-1403) was found to possess less cytotoxic activity against all the three cancer cell lines while the compound having *N*-ethyl piperazine subunit (i.e. DNJ-1404) was found to possess moderate cytotoxic activity against stomach and breast cancer cell lines and less cytotoxic activity against lung cancer cell line.

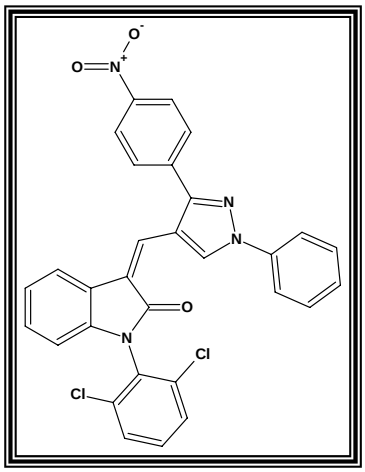
In case of DNJ-1501 to DNJ-1505, DNJ-1501 was found to possess less cytotoxic activity against all the three cancer cell lines. DNJ-1502 was found to be good active against stomach cancer cell line with the ED_{50} value 39 µg/ml while it was found to be less active against rests of the two cancer cell lines with the ED_{50} value more than 100 µg/ml. DNJ-1503 and DNJ-1504 were found to possess less cytotoxic activity against stomach (ED_{50} : >100 and 89 µg/ml respectively) and lung cancer (ED_{50} : >100 µg/ml) cell lines while they were found to possess moderate cytotoxic activity (ED_{50} : 81 and 61 µg/ml respectively) against breast cancer cell line. DNJ-1505 was found to be good active (ED_{50} : 30 µg/ml) against stomach cancer cell line while it was found to be moderate active against breast (ED_{50} : 50 µg/ml) and lung (ED_{50} : 84 µg/ml) cancer cell lines. Thus from above results it could be summarized that the compound having no substitution in benzothiazole subunit (i.e. DNJ-1501) found to be less active against all the three cancer cell lines. Furthermore the compound having methoxy group at 6^{th} position in benzothiazole subunit (i.e. DNJ-1502) was found to be good active against stomach cancer cell line while it was found to be less active against rests of the two cancer cell lines. Moreover the compounds having halogen group (i.e. Cl and F) at 6^{th} position in benzothiazole subunit (i.e. DNJ-1503 and DNJ-1504) were found to possess less cytotoxic activity against stomach and lung cancer cell lines while they were found to possess moderate cytotoxic activity against breast cancer cell line. The compound having nitro group at 6^{th}

position on benzothiazole subunit was found to be good active against stomach cancer cell line while it was found to be moderate active against breast and lung cancer cell lines.

Further study on other molecules is under progress.

6.3 IN SILICO STUDY FOR DNJ-701

In silico study for the indolinone derivatives, synthesized in Chapter – 3 (i.e. DNJ-701 to DNJ-705) and their anticancer activity was carried out at Gujarat Cancer Research Institute, (GCRI) Ahmedabad, which has been included in this chapter, while another coumarin derivatives synthesized in the same chapter are under investigation for their antitubercular and antiviral activity.



$C_{30}H_{18}Cl_2N_4O_3$

Under this title, we are focusing on some advanced comparison data of molecule for possible risk assessment and for further development and conclusion.

1 – Toxicity Risk assessment

2 – logs Calculation

3 – Molecular Weight

4 – Drug likeness

5 – Drug Score

6.3.1 TOXICITY RISK ASSESSMENT

Recent high-profile drug withdrawals increase the pressure on regulators and the pharmaceutical industry to improve preclinical safety testing. Understanding mechanisms of drug toxicity is an essential step toward improving drug safety testing by providing the basis for mechanism-based risk assessments. Nonetheless, despite several decades of research on mechanisms of drug-induced toxicity and the application of various new technologies to preclinical safety assessment, the overall impact on preclinical safety testing has been modest. Assessing the risk of exposing humans to new drug candidates still depends on preclinical testing in animals, which in many, but not all cases, predicts outcomes in humans accurately. The following offers a perspective on the challenges and opportunities facing efforts to improve preclinical safety testing and outlines gaps and needs that must be addressed. A case is built for focusing solutions on defined problems within the current safety testing paradigm rather than imposing wholesale change. Targets for application of new technologies are available now. Improving drug safety testing will depend on improving the application of mechanism-based risk assessment but will also require improving public and private collaborations in order to focus research regarding the mechanism of drug-induced toxicity on the most important problems.

Before doing the toxicity prediction we ran a set of toxic compounds and a set of presumably non-toxic compounds through the prediction. The data below shows the results obtained by predicting all available structures of four subsets of the database. E.g. all structures known to be mutagenic were run through the mutagenicity assessment. 86 % of these structures where found to bear a high or medium risk of being mutagenic. As a control set served a collection of traded drugs of which the mutagenicity risk assessment revealed only 12 % of potentially harmful compounds.

Results of given substance, which obtain from one of those kind of procedures are mentioned.

Risk of mutagenicity		
Result	1.0	no risk
Mutagenicity of currently available drugs		
No. of Drugs	3343	
High risk	09 %	
Medium risk	03 %	
Low risk	88 %	

Risk of Tumorigenicity		
Result	1.0	no risk
Tumorigenicity of currently available drugs		
No. of Drugs	3343	
High risk	05 %	
Medium risk	01 %	
Low risk	94 %	

Risk of Irritating effect		
Result	1.0	no risk
Irritating effect of currently available drugs		
No. of Drug	3343	
High risk	04 %	
Medium risk	04 %	
Low risk	92 %	

Risk of reproductive effect		
Result	1.0	no risk
reproductive effect of current available drug		
No. of Drug	3343	
High risk	09 %	
Medium risk	01 %	
Low risk	90 %	

(The values are 1.0, 0.8 and 0.6 for *no risk*, *medium risk* and *high risk*, respectively.)

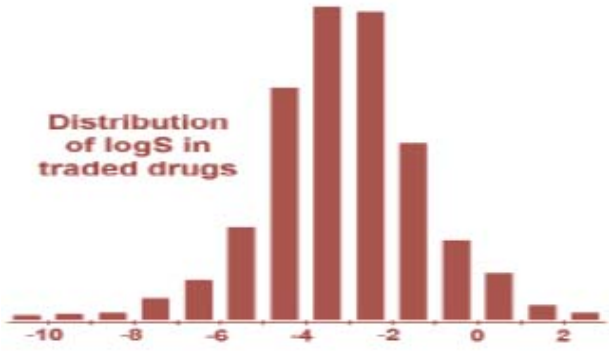
The prediction process relies on a precomputed set of structural fragment that give rise to toxicity alerts in case they are encountered in the given structure. These fragment lists were created by rigorously shreddering all compounds of the database known to be active in a certain toxicity class (e.g. mutagenicity). During the shreddering any molecule was first cut at every rotatable bonds leading to a set of core fragments. These in turn were used to reconstruct all possible bigger fragments being a substructure of the original molecule. Afterwards, a substructure search process determined the occurrence frequency of any fragment within all compounds of that toxicity class. It also determined these fragment's frequencies within the structures of more than 3300 traded drugs. Based on the assumption that traded drugs are largely free of toxic effects, any fragment was considered a risk factor if it occurred often as substructure of harmful compounds but never or rarely in traded drugs.

6.3.2 logS Calculation

Unfortunately, traditional methods used to measure solubility are neither rapid nor cost effective for higher throughput screening. As a result, solubility measurements are often pushed down in development process where the numbers of active compounds are significantly reduced. As a result, direct experimental solubility data is not available to assess of the scope of the overall solubility profile of the drug candidates being produced. Although there is no general consensus among drug researchers that solubility measurements (at early screening phases) are necessary to improve the overall a drug space' that compound libraries occupy, there is a common appreciation of the need for faster, more affordable solubility measurements to improve the drug discovery process as a whole.

The aqueous solubility of a compound significantly affects its absorption and distribution like characteristics. Particularly, a low solubility goes along with a bad absorption and therefore the general aim is to avoid

poorly soluble compounds. Our estimated logS value is a unit stripped logarithm (base 10) of the solubility measured in mol / liter. In the left diagram you can see that more than 80% of the drugs on the market have a (estimated) logS value greater than -4.



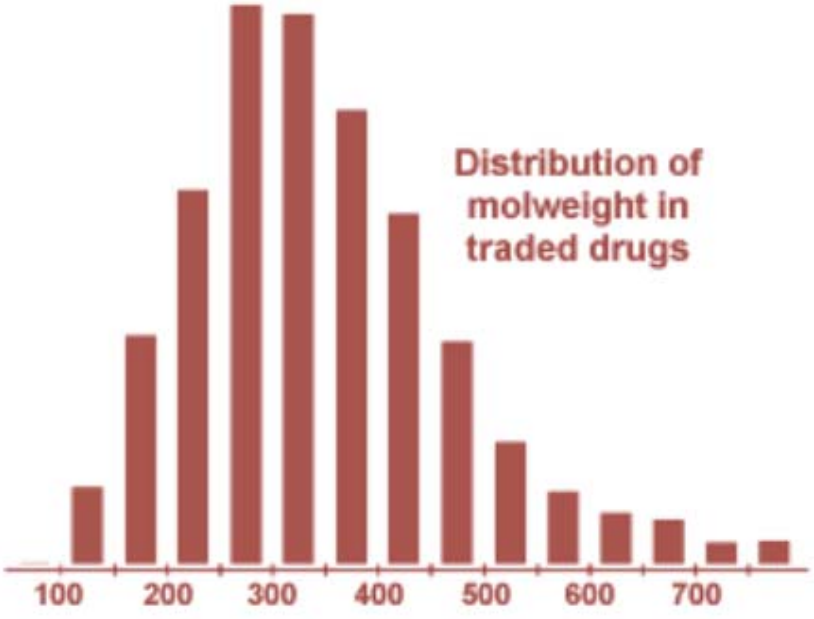
Result of $C_{30}H_{18}Cl_2N_4O_3$ logS

Base value	- 0.530
1 * 0.016 Atom type	0 x 41404
2 * -0.315 Atom type	0 x 60809
1 * -0.05 Atom type	0 x 80804
1 * 0.192 Atom type	0 x 81404
16 * -0.181 Atom type	0 x 8220802
1 * -1.066 Atom type	0 x 8520802
1 * 1.327 Atom type	0 x 8520803
1 * 0.611 Atom type	0 x 20260802
4 * -1.317 Atom type	0 x 4088220802
1 * -0.849 Atom type	0 x 4148220802
2 * -0.602 Atom type	0 x 4248220802
1 * -1.681 Atom type	0 x 6088220802
1 * -1.258 Atom type	0 x 6088520802
1 * 1.07 Atom type	0 x 6088520803
1 * 1.933 Atom type	0 x 6098240802
1 * 1.109 Atom type	0 x 8098240802
1 * 0.128 Atom type	0 x 8150240802
Result of logS	0.045

(Solubility values are estimated applying an atom type based increment system. Atom type is 64 bit numbers describing atoms and their near surrounding.)

6.3.3 Molecular Weight

Optimizing compounds for high activity on a biological target almost often goes along with increased molecular weights. However, compounds with higher weights are less likely to be absorbed and therefore to ever reach the place of action. Thus, trying to keep molecular weights as low as possible should be the desire of every drug forger. The diagram shows that more than 80 % of all traded drugs have a molecular weight below 550.



Molecular weight of C30H18Cl2N4O3 = 553.0 gm/mol

6.3.4 Drug Likeness

In the field of drug design, virtual screening is widely used for discovering novel lead candidates. By exploring virtual technology, compounds having certain activities for target would be found out efficiently. One of the most important factors for the success of virtual screening is drug likeness. Thus the reliable methodologies to construct high quality database

have been expected. It is desired that chemical structures in database exhibit drug-like properties in order to avoid problems in the later phase of drug development process. Thus statistical model for estimating drug-likeness of chemical structures has been required in order to build high quality database.

There are many approaches around that assess a compound's drug likeness partially based on fingerprints of MDL structure keys or other properties as LogS and molecular weights. Our approach is based on a list of about 5300 distinct substructure fragments with associated drug likeness scores. The drug likeness is calculated with the following score values of those fragments that are present in the molecule under investigation:

The fragment list was created by shreddering 3300 traded drugs yielding a complete list of all available fragments. As a restriction the shredder considered only rotatable bonds as cuttable. In addition the substitution modes of all fragment atoms were retained, i.e. fragment atoms that hadn't been further substituted in the original compounds were marked as such and atoms being part of a bond that was cut were marked as carrying a further substituent. This way fragment substitution patterns are included in the fragments.

The occurrence frequency of every one of the fragments was determined within the collection of traded drugs and within the supposedly non-drug-like collection of compounds. All fragments with an overall frequency above a certain threshold were inverse clustered in order to remove highly redundant fragments. For the remaining fragments the drug likeness score was determined as the logarithm of the quotient of frequencies in traded drugs.

A positive value states that your molecule contains predominantly fragments which are frequently present in commercial drugs. What it doesn't necessarily mean, though, is that these fragments are well balanced concerning other properties. For instance, a molecule may be composed of drug-like, but lipophilic fragments only. This molecule will have a high drug

likeness score although it wouldn't really qualify for being a drug because of its high lipophilicity.

6.3.5 Drug Score

The drug score value combines all other predictions into one grand total.

Score from logs	*0.045 (logs = -8.046)*
Score from moleweight	*0.351 (mol. weight = 553.0)*
Score from druglikeness	*0.0 (drug likeness = -8.587)*
No risk of Mutagenicity, Score	*1.0*
No risk of Tumorigenecity, Score	*1.0*
No risk of Irritating effects, Score	*1.0*
No risk of Reproductive effects, Score	*1.0*

(The drug score combines toxicity risks, drug likeness, logS, molecular weight in one handy value than may be used to judge the compound's overall potential to qualify for a drug. This value is calculated by multiplying contributions of the individual properties.)

6.3.6 Conclusion

With the help of above mention data and other experimental results of compound, it is clear that this compound has fulfilled most of the requirements of drug like potential and ready for future work and experiment.

SUMMARY

The work represented in the thesis entitled “Synthesis and biological profile of some novel heterocyclic moieties bearing Nitrogen, Sulphur and Oxygen atoms” is divided into six chapters which can be summarized as under.

Chapter – 1 deals with the 2-methyl indoline derivatives. Being an analogue of indole nucleus, 2-methyl indoline is having diversified biological profile. It was decided to explore new chemistry involving 2-methyl indoline moiety and to carry out their biological evaluation.

Synthesis and yield optimization of the 2-methyl indoline was carried out in **Chapter – 1**. Literature survey revealed that different types of *N*-alkylation reactions have been carried out on 2-methyl indoline but only one reaction is reported for Mannich reaction on 2-methyl indoline, where benzotriazole was used as a secondary amine along with formaldehyde (37-41% w/w solution). So looking to the unreported chemistry aspect, different secondary amines and primary aromatic amines were selected to carry out Mannich reaction on 2-methyl indoline in order to acquire *N*-Mannich bases.

Importance of microwave irradiation in organic synthesis has been described in **Chapter – 2**. **Chapter – 2** is also dealing with different 2-methyl indoline derivatives and isatin derivatives. Literature survey revealed that *N*-alkylation with the chain having three carbon atoms and different secondary amines at another end has not been reported using indoline and isatin nucleus so far. Thus *N*-alkylation with different alkyl chains and their biological evaluation was the aim behind the work done in this chapter.

The synthesis of pyrazoles remains of great interest owing to the wide applications in pharmaceutical for their analgesic, antipyretic, antibacterial and anti-inflammatory properties. **Chapter – 3** is consisting study of different types of reactions involving pyrazole aldehyde as a chemically reactive component. In this chapter, five different pyrazole aldehydes were synthesized and reacted with 1-(2, 6-dichlorophenyl)-2-indolinone, 3-acetyl-4-

hydroxycoumarin, 4-hydroxycoumarin with and without urea / thiourea and their condensation products were studied.

Chapter – 4 deals with the different type of newly synthesized arylaminocoumarin derivatives. Many methods have been reported for the synthesis of arylaminocoumarins. Literature survey reveals that in 1982, Savel'ev *et. al.* reported novel arylaminocoumarin derivatives. They prepared *C*-Mannich bases of 4-hydroxy coumarin followed by chlorination at C_4 position and their condensation with different aromatic primary amines. It was interesting to study the results of the Mannich reaction on arylaminocoumarins. Different types of arylaminocoumarins were synthesized by adopting microwave assisted method and Mannich reaction was carried out on it. Study of this reaction was of interest due to the presence of two active sites in the molecule for Mannich reaction. Mannich reaction goes on the atom having hydrogen of pronounced activity irrespective of carbon or nitrogen. In this case, due to the tautomerism under acidic conditions, C_3 position of coumarin nucleus became more reactive than the secondary nitrogen and it resulted into unexptected products. To confirm the action of Mannich reagents, single crystal of one of the derivative was developed which clearly shows that the compounds synthesized in this chapter are the *C*-Mannich bases and are not the *N*-Mannich bases.

2-Indolinone derivatives have recently been established as an anti cancer compounds and more specifically as tyrosine kinase inhibitors (*SU5416*, *SU5614*, *SU6668*, *SU6597*, *SU6663* and *SU6561*) that block kit activation and growth of small cell in lung cancer. Thus the preparation of different 2-indolinone derivatives was carried out in **Chapter – 5**. Three different schemes were included in this chapter having isatin and Schiff base as core moiety. Schiff bases of isatin were prepared using 2-propylpentanohydrazide and 2-aminobenzothiazole which subsequently underwent Mannich reaction using different secondary amines and formaldehyde (37-41% w/w solution). In another approach, Schiff bases were prepared using isatin and differently substituted 2-aminobenzothizoles which subsequently underwent for *N*-acetylation in isatin nucleus.

Chapter – 6 deals with the biological evaluation of newly synthesized compounds. In this chapter, antimicrobial activity of 41 compounds synthesized in Chapter – 1 and Chapter – 2 is reported. Furthermore, in silico study of *N*-substituted indolinone derivatives synthesized in Chapter – 3 is described. This chapter also includes cytotoxic activity data of the 15 compounds synthesized in Chapter – 5, while rests of the compounds are under investigation.

CONFERENCES/SEMINARS/WORKSHOPS ATTENDED

1. National Workshop on Management and use of chemistry databases and patent literature during February 27th to 29th – 2008 jointly organized Department of Chemistry, Department of Computer Science, Computer Centre-Saurashtra University and Gujarat Council on Science & Technology (GUJCOST)-Gandhinagar held at Rajkot.

2. International Conference On The Interface Of Chemistry-Biology In Biomedical Research during February 22nd to 24th – 2008 jointly organized by I.S.C.B. and Chemistry Group, Birla Institute of Technology & Science, Pilani held at Pilani. (Paper presented)

3. National Seminar on Recent Advances in Chemical Science & An Approach to Green Chemistry during October 11th to 13th – 2006 jointly organized by Department of Chemistry, Saurashtra University & Gujarat Council On Science & Technology (GUJCOST)-Gandhinagar held at Rajkot.

4. National Workshop on e-Resources in Chemical Synthesis and Natural Products during March 2nd & 3rd – 2006 organized by Department of Chemistry, Saurashtra University held at Rajkot.

5. National Workshop on Nanotechnology: Opportunities & Challenges on October 17, 2005 jointly organized by Saurashtra University-Rajkot & Gujarat Council On Science & Technology (GUJCOST)-Gandhinagar held at Rajkot.

6. Regional Workshop-An approach to Analytical Instruments Commonly Used in Chemical Industries during September 17th to 18th – 2005 organized by Department of Chemistry, Bhavnagar University held at Bhavnagar.

7. 9th National Conference on Bioactive Heterocycles & Paradigm shift (Including one day International Symposium on Drug Discovery –

Recent Advances) during January 8th to 10th – 2005 Organized by I.S.C.B. & Department of Chemistry held at Saurashtra University – Rajkot.